Nutritional Limits of Gigantism

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Allometry of Digestive Anatomy and Physiology in Herbivores with Special Reference to Methane Losses

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Contents

Summary

An evolutionary increase in body mass has often been considered to be linked with advantages in several terms. One prominent concept is that of an increasing digestive efficiency in larger herbivores, which has found widespread application in ecology. The so-called Jarman-Bell principle suggests that larger herbivores have digestive advantage due to allometric principles. This concept is based on a discrepancy between the allometric scaling of gut capacity and gut fill rate (food intake rate). Metabolic requirements and hence the daily food intake are generally a function of their body mass raised to the power of 0.75, whereas the gut capacity scales linearly, i. e. to a higher exponent (1.00). Therefore, more gut capacity per unit food intake is available with size increase, which might result in a longer ingesta retention time, with increasing body mass. As fermentation in herbivores is a time-dependent factor, longer retention times have been linked with higher digestive efficiency. The Jarman-Bell principle suggests that larger animals can subsist on a diet of lower quality (because a longer MRT allows a more thorough digestion), while small-bodied animals are constrained to feed on higher quality items (low in fibre) due to their relatively high metabolism and lower relative gut capacity.

The results of this study indicate that gut capacity, measured as wet contents of the gastrointestinal tract, scales nearly isometrically with body mass ($BM^{1.00}$) and daily food intake scales about the power of 0.75 in reptilian and mammalian herbivores. These results support the considerations of the Jarman-Bell principle. In contrary to the general assumption, less scaling of ingesta retention and digestive efficiency with body mass was found in herbivorous reptiles and mammals. These results imply no advantage with size increase.

Even disadvantages are associated with increase in body mass, such as ingesta particle size and potentially methane production. The results of this study suggest that methane output, in a broad-scale comparison, scales linearly with body mass in reptilian and mammalian herbivores across a large range of body mass. This translates into an increase of energy losses due to methane as a proportion of overall energy intake with body mass. In methane production ruminant species reached the highest level, followed by non-ruminant mammalian herbivores, which had similar levels as reptilian herbivores. The scaling of methane production with body mass adds to the assumption that, contrary to previous concepts, an increase in body mass does not necessarily translate into a digestive advantage. Whatever the causes of the increased methane output in ruminants are, its scaling with body mass may be responsible for the different body mass ranges achieved by ruminant and non-ruminant herbivores and thus represent an intriguing example of a physiological constraint on the evolutionary history of a particular animal group.

Zusammenfassung

Ein Wachstum der Körpergröße wurde häufig mit verschiedensten Vorteilen assoziiert. Ein sehr bekanntes Konzept ist das der zunehmenden Verdaulicheffizienz bei Pflanzenfressern zunehmender Körpergrösse, das weit verbreitete Anwendung in der Ökologie gefunden hat. Dieses so genannte Jarman-Bell Prinzip sagt aus, dass größere Pflanzenfresser Vorteile im Bezug auf die Verdauung haben auf Grund von allometrischen Effekten. Dieses Prinzip basiert auf zwei Überlegungen: Das Darmvolumen skaliert linear (mit dem Exponenten 1.00) mit der Körpergröße, wohingegen die Futteraufnahme, entsprechend des Energiebedarfs, zu einem geringeren Exponenten, 0.75, skaliert. Das würde bedeuten, dass mit zunehmender Körpergröße mehr Darmvolumen pro Einheit Futteraufnahme zur Verfügung stehen würde; was wiederum zu längeren Passagezeiten des Nahrungsbreis führen könnte. Da Verdauung von Pflanzenfasern zeitabhängig ist, werden höhere Verdaulichkeiten bei verlängerter Passagezeit vermutet. Das Jarman-Bell Prinzip postuliert daher, dass größere Tiere sich von Nahrung niedriger Qualität ernähren können (weil längere Passagezeiten eine gründlichere Verdauung erlauben), während kleinere Tiere dazu gezwungen sind, qualitativ hochwertige Nahrung zu sich zu nehmen auf Grund ihres hohen Stoffwechsels und verhältnismässig geringeren Darmvolumens.
Die Ergebnisse der folgenden Studien zeigen, dass gemäß der Annahme des Jarman-Bell Prinzips das Darmvolumen linear (Körpermasse$^{1.00}$) und die Futteraufnahme bei Pflanzen fressenden Reptilien und Säugern ungefähr mit der metabolischen Körpermasse (Körpermasse$^{0.75}$) skaliert. Entgegen der allgemeinen Annahmen wurde weder für die mittlere Passagezeit, noch für die Verdaulichkeit eine positive Korrelation der Körpermasse gefunden. Diese Ergebnisse implizieren, dass steigende Körpermasse keinen Vorteil im Bereich der Verdauungsphysiologie erbringt.
Mit ansteigender Körpergröße sind zudem auch Nachteile verbunden, wie zum Beispiel ansteigende Partikelgröße des Nahrungsbreies und möglicherweise die Methanproduktion. Die in dieser Arbeit vorgelegten Ergebnisse lassen vermuten, dass Methanproduktion in einem weit reichenden Vergleich linear mit Körpergröße (Körpermasse$^{1.00}$) bei Pflanzen fressenden Reptilien und Säugern skaliert. Das stellt einen ansteigenden Energieverlust in Relation zur Gesamtenergieaufnahme auf Grund von Methanproduktion mit ansteigender Körpergröße dar. Die höchste Methanproduktion wurde bei Wiederkäuern gefunden, gefolgt von den nicht wiederkäuenden Säugern, die wiederum eine vergleichbare Produktion wie Pflanzen fressende Reptilien aufwiesen. Die Korrelation von Methanproduktion mit der Körpermasse unterstützt die Annahme, dass entgegen früherer Annahmen ein Anstieg der Körpergröße nicht zwangsläufig zu einem Vorteil in der Verdauungsphysiologie. Was auch immer der Grund für der erhöhte Methanproduktion bei Wiederkäuern sein mag, die Korrelation mit Körpergröße könnte für die unterschiedlichen Körpergrößenbereiche verantwortlich sein, die von Wiederkäuern und nicht wiederkäuenden Pflanzenfressern erreicht werden und damit ein interessantes Beispiel für eine physiologischen Limitierung in der evolutionären Geschichte bestimmter Tiergruppen darstellen.

Introduction

Body mass

Body mass (BM) is an important factor not only in physiology, but also in terms of evolutionary biology. Organisms vary in BM by more than 21 orders of magnitude, from bacteria (10^{-13}g) to whales (10^{8}g) (Karasov & Martínez del Rio 2007). Due to this, biological diversity has been announced to be largely a matter of size (Brown et al. 2000). Furthermore BM has been exclaimed as the most important attribute of an animal, both physiologically and ecologically (Bartholomew 1981).

The tendency for organisms in evolving lineages to increase in size over time has been stated as Cope's rule (Kingsolver & Pfennig 2004; Hone & Benton 2005). In evolutionary terms, large BM has been considered as an advantage, e.g. to enhance the ability to avoid predators and catch prey (Alroy 1998). Hone and Benton (2005) mentioned positive effects of large BM like increased defence against predation, increased success in intraspecific competition, as well as problems caused by large BM, like increased development time and increased demand for resources. A trend towards larger BM within the same lineages has been documented for large North American mammals, for both herbivores and carnivores (Alroy 1998).

Allometry

Many traits that have been linked to BM vary with BM in a predictable fashion, and this predictability is fundamentally useful because it allows to summarize and compare data (Karasov & Martínez del Rio 2007). The relationship between an organisms BM and another of its characteristics (Y) is often to be well described by a power function $Y = a\ BM^{b}$. This is called a power function because the dependent variable (Y) changes as some power to the independent variable. The relation between Y and BM can have very different shapes depending on the value of b, e.g. if b=0, BM has no influence on Y, if b=1, this means that Y scales linearly with BM. Because BM and Y increase at different rates, these relations are often called 'allometric relations' and power functions involving BM 'allometric equations' (allos: other; metron: measure)(Peters 1983). As linear relations are much easier to manipulate than power functions, allometric equations are frequently converted to their logarithmic form. In doing so, log-log plots

include a more even spread of data set across the axes and the ability to plot a wide range of BM and Y values (Karasov & Martínez del Rio 2007). In spite of their usefulness, log-log transformations must be used with caution, because fairly large absolute differences can appear small on log-log scales. However, in fact the data remain unchanged by transformation. Karasov and Martínez del Rio (2007) summarized the most important benefits derived from allometric relationships: 1) extremely large data sets can be summarized in them; 2) one can use them to make allometric 'educated guesses'; 3) one can use them to derive new relationships and to formulate theoretical expectations; and 4) allometric laws allow us to compare organisms of different sizes. Therefore, allometry remains an essential tool in the analytical arsenal of different biological traits.

Digestion in herbivores

For the development of very large BM in terrestrial vertebrates an herbivorous feeding habit seems to be a prerequisite, due to the higher energy available for a population on the low trophic level of primary consumers. Therefore, within ecosystems, the largest herbivores usually attain BM that excel those of carnivores by magnitudes (Burness et al. 2001).

In principle, organisms that consume a plant-based diet face one important problem: While cell constitutes can be digested directly by vertebrates, cell walls usually cannot because they consist of pectin and fiber (cellulose, hemicellulose and lignin). Therefore, extant reptilian, mammalian and avian herbivores rely on a symbiotic gut microflora to digest plant fibre. Anatomically specialized sections of the gastro-intestinal tract often are set aside as so-called fermentation chambers to provide the microflora with favourable conditions like an anaerobic environment, appropriate temperature, an alkaline or neutral pH, continuous and appropriate nutrient and fermentation substrate supply. Among vertebrate herbivores, two principal sites for fermentation chambers are known (Stevens & Hume 1995). These fermentation chambers can be located anterior to the acid stomach (in ruminants and foregut fermenters) or posterior to the stomach and small intestine (in hindgut fermenters). The hindgut fermentation chamber may either be located primarily in the colon or in the caecum – such as in the paired caeca in birds like ostrich (*Struthio camelus*) and grouse (*Dendragapus obscurus*), or the caeca of rodents (like the capybara *Hydrochoerus hydrochaeris*, guinea pig *Cavia porcellus*) or lagomorphs (like rabbits

Oryctolagus cuniculus). In the latter (mammalian) taxa, the strategy of hindgut fermentation is coupled to coprophagy, allowing the animals to make use of the microbial protein built up in the gut (Hummel & Clauss 2010).

During fermentation, enzymes produced by microbes (bacteria, archaea, fungi and protozoa) degrade structural carbohydrates of plants (like cellulose, hemicellulose, pectin), producing the so-called "short-chained fatty acids" (SCFA). Methanogenic microorganisms – the archeae – are part of the microbial fauna in the fermentation chambers of the gastrointestinal tracts of herbivores (Stevens & Hume 1998). Archeae act as hydrogen (H_2) sinks, converting H_2 and CO_2 to methane, thus keeping the partial pressure of H_2 low, which enhances the activity of other fermenting microorganisms in the gut ecosystems (Jensen 1996). The principle products of fermentation include SCFA, methane and CO_2. While the SCFA are the principle source of energy available for the vertebrate host (Stevens & Hume 1998), methane is an energy sink that is passed as a gas. Methane production is an unavoidable side-effect of herbivory in vertebrates and has been demonstrated in the faeces of nearly all herbivorous and additionally some omnivorous and carnivorous terrestrial vertebrates (Hackstein & Van Alen 1996).

A potential explanation for a lower methane production in the hindgut, when compared to the rumen, has been stated by Prins and Lankhorst (1977), Jensen (1996) and Immig (1996). These authors speculate that other hydrogen sink mechanisms than methane production are involved in removing hydrogen from the hindgut. Reductive acetogenesis, another hydrogen sink which converts H_2 and CO_2 to acetate, has been detected in the hindgut of rodents (Prins & Lankhorst 1977) and ostrich (Fievez et al. 2001). However, Fievez et al. (2001) determined methane in the lower colon of ostriches as well. So far, reductive acetogenesis has not been detected in the rumen, although acetogenic bacteria has were found at this site (Immig 1996). However, reductive acetogenesis is unlikely to rule out methanogenesis completely; on the contrary, in vitro studies indicate that in competition between acetogenic bacteria and methanogens for hydrogen methanogens are predominant (Gibson et al. 1990).

Energy budget

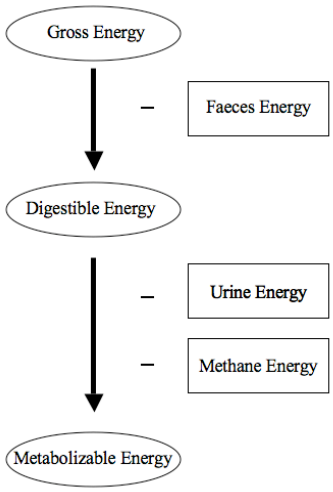

Fig. 1: Energy budget

The energy gains of herbivores depend on the food intake and the metabolizability of the diet.

When measuring the digestibility of a diet, it is hard to distinguish between endogenous losses (metabolic, microbial, secretion) in the faeces and the undigested residual of the food. The digestible energy is the gross energy of the ingested food minus the energy lost in faeces (Fig.1). If one subtracts urine and methane energy from the digestible energy, the more important factor, the metabolizable energy, is the result (Fig.1). In other words metabolizable energy is the proportion of food material actually available for the organism.

Diet quality

A diet high in fibre has been postulated to be low-quality food. Demment and Van Soest (1985) considered that plant abundance is positively correlated with a high cell wall content, which means that high-quality food is rare and low-quality food is common. Therefore, to maintain a full gut for continuous processing, larger herbivores must use forage of lower quality. Gut capacity, food intake and ingesta passage are considered to be pure “animal factors”, which are allometrically related to, and therefore determined by, BM (Clauss et al. 2007). If gut capacity is a constant fraction of BM, then an increase in food intake might lead to a faster ingesta passage. However, some factors limiting nutrient gain in herbivores have been discussed. For example, the degree of selectivity (and therefore diet quality) is said to decline with BM due to a limited time budget (Demment & Van Soest 1985). A diet high in quality (fruits, seeds, flowers, sprouting shoots) represents only a tiny fraction of the total plant and is therefore only available in small amounts, and the absolute ingested amount of such high-quality feeds is much too low to meet the quantitative daily requirements of large herbivores (Geist 1974; Demment & Van Soest 1985).

Digestive efficiency

The digestive efficiency depends on the quality, retention time, and the particle size of the ingested food. As digestion of fibrous diets is a slow process, the mean retention time (MRT) of the ingested food has to be long enough for the microorganisms to break down the fibre. Longer MRT have been suggested to be linked to higher digestive efficiency, because the ingesta is longer available for the fibre-fermenting microbes (Udén & Van Soest 1982; Clauss et al. 2007). In the trade-off between digestive efficiency and ingesta retention time, case is difficult to separate from effect. A nice example is mentioned in McNab (2002): howler monkeys feed principally on leaves, supplemented with fruits and have long passage times (16-24h), whereas spider monkeys that feed principally on fruits, which they supplement with leaves, have short passage times (4-5h). Does the physiological adaptation determine the diet chosen by the animals, or does the diet - chosen by the animals for other reasons - influence the physiological measurement?

Jarman-Bell principle

Such considerations were subsumed in one principle. Geist (1974) explained how two PhD studies, which resulted in two publications (Jarman 1968; Bell 1971), crystallized an understanding of the ways in which BM affects the ecology of ungulates of the African savannas. These studies have become the springboard for most analyses of ecological interactions that include a range of BM (du Toit 2005). The so-called 'Jarman-Bell principle' (JBP) (Jarman 1968; Bell 1971; Geist 1974) suggests that large body size is a digestive advantage in mammalian herbivores.

An increase in ungulate BM is associated with an increase in dietary tolerance (Jarman 1968; Bell 1971; Geist 1974; Jarman 1974; Demment & Van Soest 1985). Increased dietary tolerance (measured in terms of the range in fibre content of the herbage the animal can tolerate as food) results from the nutritional advantages that accrue to large herbivores through: (1) decreased mass-specific metabolic demands (as daily energy requirements are related to metabolic body mass, $BM^{0.75}$). For this reason, small-bodied species require more energy per day per unit of BM than do large-bodied forms (Geist 1974). Although larger species also prefer high-quality food, their absolute daily intake requirements force them to accept more abundant food of lower

quality, which they can tolerate. Jarman (1974) showed that in East African antelopes, food choice involves the quality and abundance of foods and is associated with BM, group size and social structure. While small antelopes selected high-quality food, larger ones used abundant, low-quality foods.

Including another digestive parameter, gut capacity, has extended the JBP. Gut capacity has been suggested to be a constant fraction of BM (Demment & Van Soest 1985; Parra 1987; Woolnough & du Toit 2001; Clauss et al. 2007). If gut capacity effectively scales to $BM^{1.00}$, and food intake scales to $BM^{0.75}$, these different scaling effects result into a larger gut capacity per unit food intake with increasing BM. This again should in theory lead to an increase in MRT of the ingested food with increasing BM (Illius & Gordon 1992; Robbins 1993; Clauss et al. 2009)(Fig.2).

Hummel and Clauss (2010) summarized the relationship between these three parameters:

"*Given the two relationships of*

gut capacity ~ body mass$^{1.00}$

and

food intake ~ body mass$^{0.75}$

it can be concluded theoretically that the time food stays in the gut (the ingesta passage or ingesta retention time) scales to body mass$^{(1.00-0.75)}$ *or body mass*$^{0.25}$.

This concept has been used to explain or claim that

- *larger herbivores can use food of lower quality (because a longer retention time allows a more thorough digestion)*
- *on similar diets, larger herbivores achieve higher digestibilities (because the same diet is exposed to a longer digestion time)."*

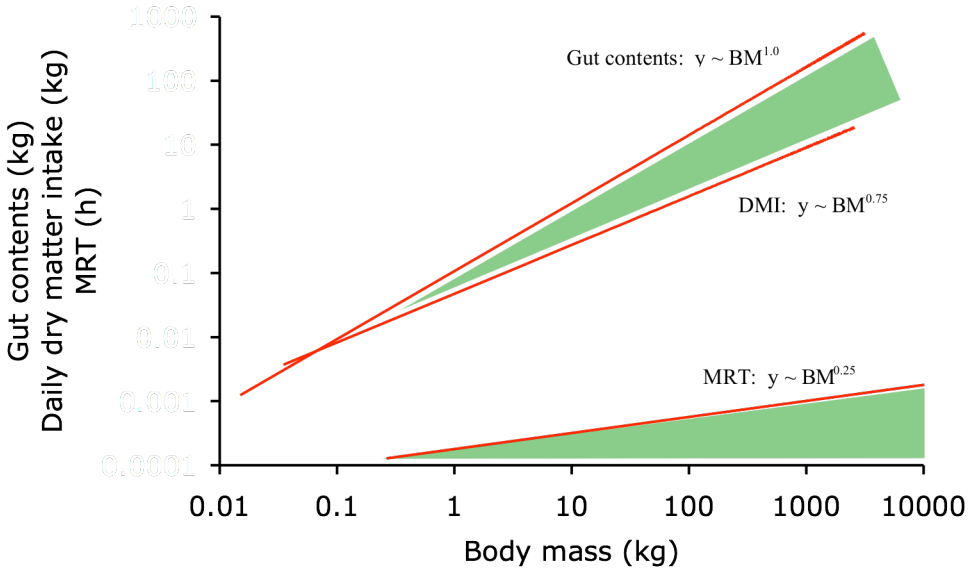

Fig. 2: Scaling of gut contents, daily dry matter intake (DMI) and ingesta mean retention time (MRT) with body mass.

General discussion

Prominence of the Jarman-Bell principle

The JBP (Jarman 1968; Bell 1971; Geist 1974) is maybe the most prominent set of allometric considerations in the field of herbivore digestive physiology, and it has been cited frequently (4000 citations, 'Jarman-Bell, principle' source: www.scholar-google.com), referring to different aspects:

1) Size-related differences in food quality (1010 citations, 'Jarman-Bell principle, food quality')

Comparing related species the JBP predicts a negative correlation between BM and overall diet quality (Gaulin & Sailer 1985; Mysterud 2000; Agetsuma 2001; Woolnough & du Toit 2001; Campos-Arceiz et al. 2004).

2) Sexual differences in diet use (166 citations, 'Jarman - Bell principle, size dimorphism')

Within a sexually size-dimorphic herbivore species, the JBP predicts that adult males and females will differ in feeding ecology, with larger males accepting diets higher in fibre. In terms of sexual differences in dietary choice, this prediction from the JBP received theoretical support (Illius & Gordon 1987; Pérez-Barberìa & Gordon 1998; Conradt et al. 1999; Barboza & Bowyer 2000; du Toit 2005).

3) Ingesta retention time (563, 'Jarman - Bell principle, retention time')

As gut capacity is assumed to scale linearly to BM, and dry matter intake (DMI) to metabolic body mass ($BM^{0.75}$), and therefore more gut capacity per unit food intake would be available, it has been proposed that ingesta MRT should be positively correlated to BM (Milton 1984; McNaughton & Georgiadis 1986; Lambert 2002; Makhabu 2005; Clauss et al. 2007).

4) Digestibility (342, 'Jarman - Bell principle, digestibility')

Furthermore, if BM of an animal relates to gut volume and retention time of food, BM might affect the extent of digestion of the diet (Cromsigt et al.; Schuette et al. 1998; Makhabu 2005).

Empirical data for the Jarman-Bell principle

However, although the JBP is mentioned very frequently in connection with these concepts, empirical data investigated to test the theory of the JBP are rare.

The pattern, that smaller animals selected high-quality food, whereas larger ones used abundant, low-quality foods, has been shown for primates (Crook & Gartland 1966; Agetsuma 2001; Nakagawa 2003), bats (Fleming 1991), ungulates (Hanley 1984; Owen-Smith 1988; Codron et al. 2007b; Yoshihara et al. 2008) and rodents (Smith 1995). Within the larger working group on digestive physiology of the DFG Research Group 533 on the Biology of Sauropod Dinosaurs, of which the present thesis is also a contribution, Steuer (2010) demonstrated in a comparison of free-ranging and captive ungulates that free-ranging animals ingest diets of lower overall quality with increasing BM.

Furthermore, a difference in dietary quality within sexes was found in red deer (*Cervus elaphus*), where large males moved to areas where forages are higher in fiber but more abundant (Clutton-Brock & Harvey 1977) and a similar pattern was found in African elephants (*Loxodonta africana*) (Stokke & du Toit 2000; Shannon et al. 2006). Demment (1983) found that in baboons (*Papio cynocephalus*), the larger males ate more fibrous food than smaller females.

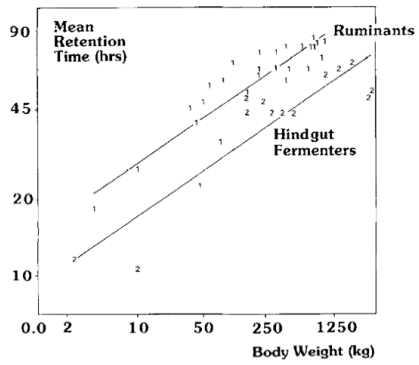

Fig. 3: Relationship between mean whole-gut retention time (h) of particulate matter and body weight (kg) in ruminants (1) and hindgut fermenters (2) (Illius and Gordon 1992).

Because of the prominence of the digesta retention time, numerous studies have investigated this parameter (Clauss et al. 2007). Illius and Gordon (1992) indicated an increase in MRT with BM (Fig.3). Their findings based on a large data set from Foose (1982) supplemented with data about smaller-bodied animals (Warner 1981).

As shown before, the JBP gained support in some extent, but some of the existing empirical data are not in agreement with the predictions of the JBP.

A principle question is whether large BM should be considered as an adaptation to low quality forage or whether low forage quality is simply a (necessary) consequence of large BM that

evolved for other reasons (Renecker & Hudson 1992). The prediction of the JBP that large-bodied herbivores are less selective and subsist on lower quality food (graminoids) than smaller ones, was not supported for ruminants by Wegge et al. (2006) and, in addition, not for grazing ruminants (Codron et al. 2007a). Interestingly, when focussing on the dataset about food quality in large African herbivores compiled by Owen-Smith (1988), a decline in food quality could not be demonstrated when animals with a BM less than 500kg are excluded. This pattern indicates that the scaling effect in dietary quality only appears in smaller herbivores (<500kg)(Hummel & Clauss 2010).
No food quality differences were found for age-related differences in BM between cows and calves (Anderson & Saether 1992). Further intraspecific comparison, including BM differences due to sexual dimorphism, could not support the JBP: Bonenfant et al. (2004) did not find support for the "forage-selection" hypothesis in red deer. In addition, no differences in feeding ecology between sexes were found in ungulates (Pérez-Barberìa et al. 2008).

The existing literature on ingesta retention times in mammalian herbivores does not unanimously support the assumption that an increase in BM is, among mammals in general, related to longer ingesta retention (Clauss & Hummel 2005; Clauss et al. 2007). As Clauss et al. (2007) showed for a large literature compilation of MRT that there is no scaling effect of this parameter with BM. This result is in contrast to those from Illius and Gordon (1992), which might be due to methodological differences between Foose (1982) and other studies (Clauss et al. 2007). If the Foose dataset is considered on its own, no scaling is evident (Clauss et al. 2009; Steuer 2010). Scaling of MRT with BM in herbivorous reptiles will be discussed in chapter 2.

In addition, empirical data on digestibility in mammalian herbivores show only poor evidence for an increase of digestive efficiency with increasing BM (Smith 1995; Pérez-Barbería et al. 2004; Pérez-Barberìa et al. 2004; Clauss & Hummel 2005; Clauss et al. 2009; Steuer 2010).
If digestibility is a scaling factor of BM will be discussed in chapter 2.

Disregarded negative effects

So far, the data rather support the concept that among large herbivores, both digestive efficiency and ingesta retention are relatively independent of BM.

The inconsistency of existing data in terms of advantages with BM might be explained by potential negative effects with size increase that have received little attention in the conceptual development of the JBP (Clauss & Hummel 2005).

In the most prominent group of extant large herbivores, the ruminants, a limitation of BM has been assumed to be associated with the completeness of fiber digestion (Demment & Van Soest 1985). Due to a scaling of retention time, an increase in BM is thought to be linked to an increased digestive efficiency. However, plant fiber cannot be digested endlessly and at the point, when fibre has been fermented completely, factors like higher gut capacity for plant material are no longer relevant. Therefore, size increase can be regarded as evolutionary profitable only as long as the parallel increasing MRT enables a corresponding increase in fiber digestion. However, as mentioned before, this digestive advantage with size increase is absent above certain BM. Anyway, no advantage with increasing BM does not represent a constraint at all and hence BM above that threshold is well possible.

The major difference between supporters and opponents of the JBP therefore is the cut-off point – whether the JBP explains variation in animals of the size of extant 'large herbivores' and 'megaherbivores' (Owen-Smith 1988), or whether it only applies to animals of a much smaller body size, such as rodents (Clauss et al. 2007).

Particle size

The bacterial fermentation of plant fiber depends not only on the time available for this fermentation, but also on the size of the ingested food particles. The smaller the particles, the more surface area is available for microbial attack; smaller particles can therefore be digested at a much faster rate as larger ones (Clauss & Hummel 2005). Therefore, it has been suggested that larger particles need to retrain longer in the gastrointestinal tract when compared to smaller particles to reach the same efficiency (Clauss et al. 2009). Furthermore, Fritz et al. (2009) mentioned that particle size reduction might explain differences in digestive efficiency that can not be explained by differences in ingesta retention time. Clauss et al. (2009) suggested that to

increase digestive efficiency, herbivores either increase digesta retention, or enhance chewing efficiency. Even if MRT might become longer with increasing BM, this 'advantage' is likely to be outweighed by the parallel increase in ingesta particle size. Thus, the theoretical assumptions on the digestive advantage of larger BM will probably not apply to a guild of herbivores that evolved adaptations for ingesta particle size reduction, and hence are subjected to a particle size allometry as demonstrated by Fritz et al. (2010) (Fig. 4).

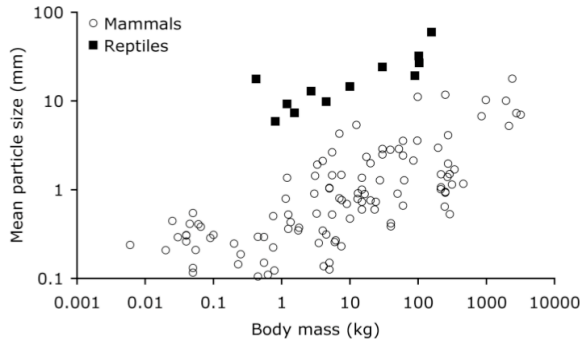

Fig. 4: Mean faecal particle size (average value per species) in non-ruminant herbivorous mammals and herbivorous reptiles across the body mass range (Fritz et al. 2010).

In contrast to mammals, herbivorous reptiles do not chew their food; and this has been mentioned as the key digestive difference between ecto- and endotherms (Fritz et al. 2009). Although herbivorous ecto- and endotherms achieve similar digestive efficiencies, endotherms do that at a faster rate, allowing a higher food intake (Karasov et al. 1986). Chewing efficiency has been considered as an adaptation to higher metabolic rates in mammals (Reilly et al. 2001; Fritz et al. 2010).

This pattern indicates that chewing reduces the time necessary for thorough fiber fermentation. Or in other words, the concept that an increased ingesta retention can compensate for a lack of ingested particle size reduction has been proposed for the comparison of reptilian and mammalian herbivores (Karasov et al. 1986). Fritz et al. showed for a large range of mammalian (2009) and reptilian (2010) species an increase in ingested particle size with BM, with a higher level in reptiles (Fig.4). Therefore, long ingesta retention times in the gastrointestinal tract and a long exposure to microbial fermentation, might well compensate for the lack of particle breakdown in herbivorous reptiles.

Methane production

Furthermore, disproportional increase of metabolic losses in faeces or via methane have been suggested as other nutritional characters that scale with BM (McCammon-Feldman et al. 1981; Van Soest 1994; Clauss & Hummel 2005).

BM limitations due to methane production have been proposed for large herbivores (Prins & Kreulen 1991; Van Soest 1994). These considerations do not refer to the methane produced by the group of fast-growing Archaea mentioned before (converting H_2 and CO_2 to methane), but to another group of slow-growing Archaea that use acetate and convert it to methane. As acetate is an important energy source for the host, this process results in reducing energy resources of the herbivore. If MRT is positively correlated with BM, the slow-growing Archaea, which have a generation time of approximately four days (Van Soest 1994), display a negative effect of size increase from a certain BM upwards. Prins and Kreulen (1991) investigated a model indicating that the maximum possible BM for ruminants would be about 1 to 1.5 metric tonnes.

Regarding long retention times (exceeding the four day limit) several vertebrate herbivores such as koalas (*Phascolarctos cinereus*), dugongs (*Dugong dugon*), sloths (*Bradypus tridactylus*) (reviewed in Clauss et al. 2007a) and land tortoises (Hatt et al. 2002), the validity of this concept might be doubted. Irrespective of this concept, a potential BM limitation or a digestive disadvantage with size increase due to the methane production of the faster-growing Archaea has not been discussed so far. There are a few studies about methane production related to BM reviewed in (Clauss & Hummel 2005). The results of the data collection indicate a possible trend for increasing energetic losses due to methane production in % of gross energy intake (Fig. 5a) and per kg dry matter intake (DMI) in ruminants (Fig. 5b).

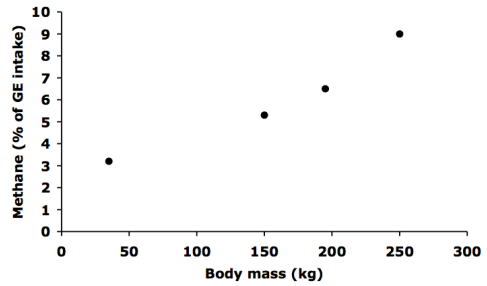

Fig. 5a. Methane losses (in % gross energy intake) as measured in different ruminant species fed lucerne hay correlated to body weight (Belyea et al. 1985; Galbraith et al. 1998).

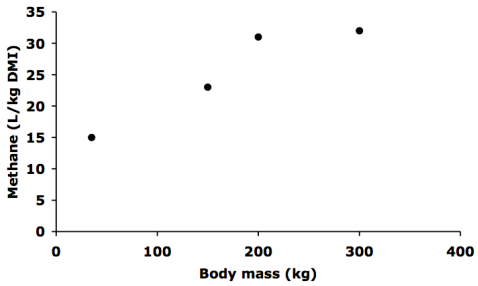

Fig. 5b. Methane losses (in litres per kg dry matter intake) as measured in different ruminant species fed lucerne hay correlated to body weight (Hironaka et al. 1996; Galbraith et al. 1998).

Although herbivorous hindgut fermenters generally harbour methanogenic archaea (Miller et al. 1986; Hackstein et al. 1996; Hackstein & Van Alen 1996), methane production is not considered to be of the same daily scope in these species as it is in ruminants (Crutzen et al. 1986). Yet, experimental data on large hindgut fermenters focus on domesticated ruminants due their contribution to the greenhouse gas effect. It has been suggested that other hydrogen sink mechanisms than methanogenesis are involved in removing hydrogen from the fermentation chambers, which might be a reason for a lower methane production in the hindgut of rodents, pigs and ruminants (Prins & Lankhorst 1977; Immig 1996; Jensen 1996) – which has been mentioned in the. Methane production as a scaling factor of BM will be discussed for mammals in chapter 3 and 4, and for reptiles in chapter 5.

Aims of research

To clarify the potential of nutritional limits to body mass in herbivores, it is essential to investigate energy losses due to digestion (such as methane production) across a large BM range in herbivores. This PhD study is part of the DFG Research Unit 533, with two further PhD students: Patrick Steuer, who studied digestive parameters within a large range of BM of herbivorous mammals (Steuer 2010), and Julia Fritz, who investigated faecal particle size as a proxy for digesta particle size in reptile, avian and mammalian herbivores (Fritz 2007). In my thesis, I focus on comparative organ allometry in reptiles, birds and mammals (chapter 1) on

food intake, digesta retention time and digestive efficiency in herbivorous reptiles (chapter 2), and especially methane production in herbivorous mammals (chapter 3 and 4) and reptiles (chapter 5). Thus, in connection with the other two dissertation theses mentioned above, we assessed aspects of allometric scaling of digestive parameters in at least two different vertebrate clades – mammals and reptiles.

The JBP has never been addressed to herbivorous reptiles before, but as the principles are independent of the *level* of the metabolic rate (one important difference between mammals and reptiles), the transfer to reptiles should be feasible. Investigating in two - in terms of digestive physiology – similar groups, the results might even be more reliable in terms of the underlying fundamental principles.

In general, tortoises and hindgut fermenting mammals are similar concerning their physiology and anatomy of digestive organs.

Adaptations in the anatomy of digestive organs are closer related to the diet than to systematic taxa. In simple-stomached herbivores, irrespective of the taxonomic classification, the gastrointestinal tract consists of the stomach, a relatively short midgut and long hindgut (Fig. 6 a+b)(Stevens & Hume 1998). As mentioned in the introduction, most herbivores have - in some cases highly - specialised region of the gastrointestinal tract, where they house their fermenting microbes. In contrast to mammalian herbivores, in which both foregut and hindgut fermenting species occur, herbivorous reptiles are known to be only hindgut fermenters. For this reason, in studying herbivorous reptiles and mammals, only hindgut fermenting mammals should be used for the direct comparison.

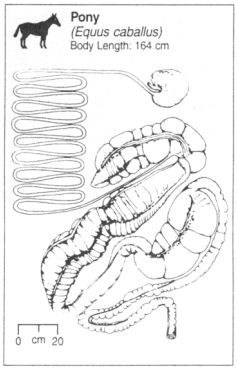

Fig. 6a: Gastrointestinal tract of a pony.

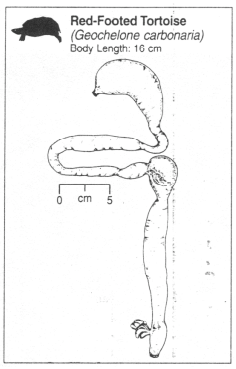

Fig. 6b: Gastrointestinal tract of a tortoise.

Outline of thesis

In my thesis, I investigate digestion in two vertebrate herbivore groups: mammals and reptiles. Based on the main question of correlation of different parameters of digestive physiology with BM, I examined potential nutritional limits to gigantism.

Chapter 1 deals with the allometry of visceral organs in amniotes. I compile allometric equations for visceral organs of herbivorous reptiles and compare them with those from the literature of mammals and birds. A visual comparison of the wet contents mass of the whole gastrointestinal tract indicates that systematic differences between herbivorous reptiles, birds and mammals are unlikely. Furthermore, estimated retention times for a 38ton sauropod would be in the same range as for extant tortoises, which do not chew their food either.

In **chapter 2**, I explore digestion in herbivorous tortoises in relation to BM. Within this group, a large range of BM can be obtained with minimal differences in digestive anatomy and physiology.

In **chapter 3**, I start with methane measurements in ruminant and hindgut fermenting mammals in relation to BM. I compare the methane production of a hindgut fermenting (Mini Shetland pony) and a ruminant (sheep) species of comparable BM fed with the same diet and add those data to a literature compilation. Higher levels of methane production in ruminants when compared to hindgut fermenting species could be corroborated, and potential limitations of BM are discussed.

In **chapter 4** I add methane data of small mammals, guinea pigs and rabbits, and analyse the correlation of methane production with BM in nonruminant mammals. The methane output of nonruminant mammals lay on the equine regression line, suggesting similarities in terms of methane production between different groups of nonruminant mammalian herbivores.

Chapter 5 deals with methane production of the other main group of herbivores, reptiles. In order to further test the concept of increasing energy losses with BM, I chose tortoises. The scaling exponent of methane production is similar in mammals and reptiles.

In parallel to the experiments necessary for the different chapters of this PhD thesis, two other studies were conducted that could be linked logistically to the same experiments:

1) No 'walking compost heaps': Core body temperature fluctuations in Giant Aldabra tortoises (*Dipsochelys dussumieri*) do not suggest relevant contribution of fermentation to body heat. (Submitted to Functional Ecology). In parallel to the feeding trials, temperature loggers could be fed to the tortoises that record the internal body temperature. It has been considered that body temperature fluctuations would decrease with increasing BM due to their thermal inertia. Furthermore, it has been suggested that in herbivorous reptiles, fermentative heat produced by fermentative microbes will contribute to a stable body temperature. Fluctuations of body temperatures, measured in the gastrointestinal tract of three large tortoises (100-180kg), obviously depended on the ambient temperature. Adding data from the literature, no effect of BM on body temperature was found when controlling for ambient temperature. Thus additional benefits in herbivores due to intestinal fermentation are unlikely in the range of body sizes studied so far.
2) Intake, selection, digesta retention, digestion and gut fill of two coprophageous species, rabbits (*Oryctolagus cuniculus*) and guinea pigs (*Cavia porcellus*), on a hay-only diet. (Submitted to *Journal of Animal Physiology and Animal Nutrition*). In parallel to the feeding trials in small mammals, digesta passage was assessed with a solute and a particle marker. The practice of coprophagy has been shown in small mammals, especially lagomorphs and rodents. A prerequisite for coprophagy is the colonic separation mechanism (CSM); two different CSM have been described - the 'wash back' of lagomorphs and the 'mucous trap' of rodents. Rabbits, with a wash-back CSM, are more efficient in extracting bacterial matter from the digesta, whereas they have a lower fibre digestibility than guinea pigs with a mucous trap CSM.

These studies are not part of this PhD thesis and are therefore only given in the appendix.

Conclusions

In contrast to the common assumption in the JBP, no evidence that an increase in BM confers a digestive advantage was found in this study. No relevant scaling in digestive efficiency and MRT with BM were found in mammalian (Clauss et al. 2007) and reptilian herbivores (this study), and therefore no advantage with size increase. Following the observations of larger herbivores feeding on a diet of lower quality (which resulted to the JBP), BM should not be considered as an adaptation to a low quality diet, but low forage quality might simply be a consequence of large BM that evolved for other reasons.

The results of the present study even suggest negative effects of increasing BM, like methane production. Because food energy intake usually scales to $BM^{0.75}$, linear scaling of methane production results into increasing energetic losses at increasing BM. This pattern is in agreement with the assumption of Clauss and Hummel (2005) that, contrary to previous concepts, an increase in BM does not necessarily translate into a digestive advantage. Furthermore, when extraploating the comparatively low level of methane production in non-ruminant herbivores at 100 metric tonnes it would correspond to 8.2-10.5 % of digestible energy intake, which is in the range of ruminants today. Therefore, if one accepts the concept that methane production represents a physiological limitation to body size evolution in ruminants, then very large sauropods could be hypothesized to have reached a similar constraint.

Another very interesting consideration resulted from this study is that the similarity between non-ruminant mammals and tortoises indicate homologies of the gut microbial fauna in ectotherms and endotherms, and that the increase in energetic losses due to methane production with increasing BM is a general allometric principle in herbivores.

Areas of further research should include further studies of methane production in non-ruminant foregut fermenters and comparisons of the microbial composition of herbivorous reptiles and mammals.

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List of abbreviations

aD	apparent digestibility
ADF	acid detergent fibre
ADL	acid detergent lignin
BM	body mass
CH_4	methane
CI	confidence interval
CO_2	carbon oxygen
CP	crude protein
CSM	colonic separation mechanism
d	day
dNDF	digestible neutral detergent fibre
DE	digestible energy
DM	dry matter
GE	gross energy
h	hour
H_2	hydrogen
JBP	Jarman-Bell principle
Kg	kilogram
L	litre
MRT	mean retention time
N	nitrogen
NDF	neutral detergent fibre
OM	organic matter
SCFA	short-chained fatty acid
SD	standard deviation

Chapter 1

Allometry of visceral organs in living amniotes and its implications for sauropod dinosaurs

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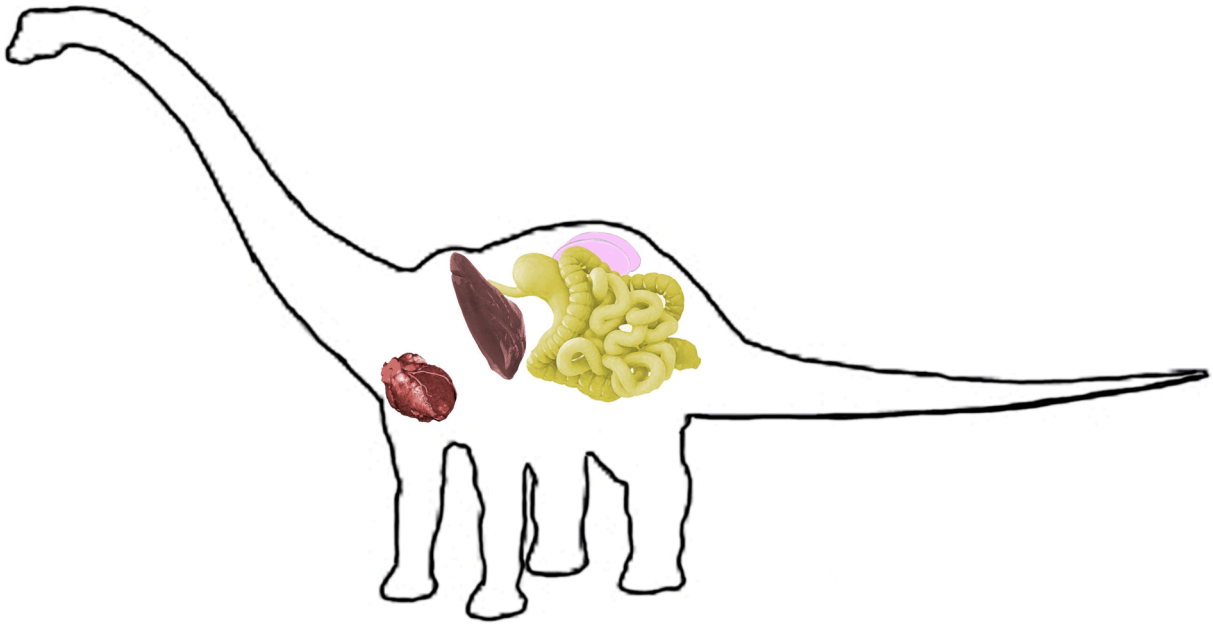

Allometry of visceral organs in living amniotes and its implications for sauropod dinosaurs

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Summary

Allometric equations are often used to extrapolate traits in animals for which only body mass estimates are known, such as dinosaurs. One important decision can be whether these equations should be based on mammal, bird, or reptile data. To address whether this choice will have a relevant influence on reconstructions, we compared allometric equations for birds and mammals from the literature to those for reptiles derived from both literature and hitherto unpublished data. Organs studied included the heart, kidneys, liver, and gut, as well as gut contents. While the available data indicates that gut content mass does not differ between the clades, the organ masses for reptiles are generally lower than those of mammals and birds. In particular, gut tissue mass is significantly lower in reptiles. When applying the results in the reconstruction of a sauropod dinosaur, the estimated volume of the coelomic cavity greatly exceeds the estimated volume of the combined organ masses, irrespective of the allometric equation used. Therefore, substantial deviation of sauropod organ allometry from that of the extant vertebrates can be allowed conceptually. Extrapolations of retention times from estimated gut contents mass and food intake do not suggest digestive constraints on sauropod dinosaur body size.

Key words: allometry, scaling, coelomic cavity, ingesta retention, digestion, gut

Introduction

Body mass is generally considered the most important predictor of morphological, physiological and ecological characteristics of animals, and a multitude of allometric correlations between body mass and other measurements have been established in biology (Peters 1983; Schmidt-Nielsen 1984; Calder 1996). While mostly used for the investigation of fundamental laws determining the functions of certain animal groups, or of life in general, allometric equations are also often used for the reconstruction of morphological, physiological and ecological traits of animals for which only body mass but few other biological parameters can be estimated directly. Especially in considerations about characteristics and constraints of the extinct dinosaur megafauna, such equations have been applied (Alexander 1989; McGowan 1989).

One interesting approach in this respect is to test whether a specific set of predictions or estimates are really compatible with other aspects of anatomy or physiology. For example, Seymour & Lillywhite (2000) demonstrated in model calculations that an upright posture of the neck in sauropods is incompatible with current understanding of cardiovascular function in vertebrates. Other examples for the use of allometry are the studies by Gunga et al. (2007; 2008), who used allometric equations on the organ size of mammals from Anderson et al. (1979), Schmidt-Nielsen (1984) and Calder (1996) to test whether reconstructions of the body size of a prosauropod and a sauropod, in particular the volume of the coelomic cavity of these animals, match the calculated space requirement of the internal organs.

For such reconstructions, a concept is required: Should physiological inferences in dinosaurs be based on mammals, birds, or reptiles, and for which parameters does this choice of extant analogue make a difference? Dinosaurs are usually considered to have been endotherms (like

birds and mammals) rather than ectotherms (reptiles), but an „intermediate“ metabolism (Reid 1997) or even a distinct ontogenetic shift in metabolic rate has been hypothesized for them (Sander & Clauss 2008), which might be relevant for the size of metabolic organs.

In order to test whether the available data suggested a difference or a similarity of allometric correlations between body mass (BM) and organ mass in reptiles, birds and mammals, we compared allometric equations for birds and mammals from the literature to allometric equations for reptiles derived from a collection of literature and hitherto unpublished data, and used the results for a plausibility test of a recent sauropod dinosaur reconstruction (Gunga et al. 2008) and a model calculation to assess whether digestive anatomy and physiology should be considered a limiting factor in sauropod body size.

Methods

A data collection on reptile organ mass was compiled using literature sources (Else & Hulbert 1981; Hailey 1997; Dohm et al. 1998), as well as unpublished data from personal observations (Hummel and Clauss, unpubl. data) and from three recent dissertation theses (Kopsch 2006; Eberle 2007; Schneemeier 2008). Data were available for the mass of the heart, the kidneys, the liver and the empty gastrointestinal tract (GIT). Data on lung tissue mass was not available from these studies, and we could not locate other sources that provided sufficient data for an inclusion of lung tissue in this study. Additionally, data on the wet content mass of the total GIT was compiled for herbivorous reptiles (Karasov et al. 1986; Parra 1978; Bjorndal & Bolten 1990; Foley et al. 1992; Barboza 1995; Hailey 1997; Mackie et al. 2004) and herbivorous birds (Herd & Dawson 1984; Dawson et al. 1989; Grajal 1995), and compared to the data collection for herbivorous mammals from Clauss et al. (2007a). If more than one set of data was available for a

species, an average was calculated and used in the analyses, in order to avoid overrepresentation of any species. The data are given in the electronic appendix.

Organ scaling is described by the allometric equation: $Y = a\ BM^b$

where Y is organ mass correlated with body mass (BM, masses in kg). The exponent *b* is a scaling factor, which describes the scaling with body size. If $b = 0$, body size has no effect; if $b = 1$, Y shows a linear correlation to BM.

Data on body mass and organ mass were ln-transformed: $\ln(\text{organ mass}) = \ln(a) + b \ln(BM)$

Linear regressions were calculated using SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA) including the 95% confidence intervals for both *a* and *b*. Because the original datasets of Calder (1996) were not available, we tested whether the 95% confidence intervals for *a* and *b* in reptiles included the values given for the respective factors and exponents for birds and mammals by this author.

Results

The 95 % confidence interval (CI) of the allometric exponent (*b*) included 1.0 for each of the four organs tested (Tab. 1); in other words, all organs did not deviate significantly from a linear correlation with body mass. The 95 % CI of the allometric exponent also included the value given by Calder (1996) for birds and mammals for the heart and kidneys (Tab. 1, Fig. 1), but not for the liver and the just not for gastrointestinal tract. The 95 % CI of the intercept of the ln-transformed equation ($\ln(a)$) included values for birds and mammals in the case of the liver, indicating that irrespective of the scaling pattern with body mass, the actual mass of this organ is similar between the three vertebrate clades in the body size range studied (Tab. 1, Fig. 1c). In the case of the heart, the mammalian value for *a* was just included in the upper 95 % CI of reptiles,

whereas that for birds was above the CI (Tab. 1, Fig. 1a). Similarly, the 95% CI for the intercept of the kidney included the mammalian but not the avian value (Fig. 1b). The reptilian intercept was lower then both the mammalian and the avian value for the gastrointestinal tract. Thus, the data indicates that the GIT of reptiles, birds and mammals shows a similar scaling pattern with body mass, but, for reptiles, at a generally lower level (Fig. 1d).

A visual comparison of data on the mass of the wet contents of the whole GIT (Fig. 2) indicates that systematic differences between herbivorous reptiles, birds and mammals are unlikely. The calculated difference in the allometric exponent between reptiles and mammals (Table 1) should therefore be viewed with caution; using the calculated equation, a reptile-like herbivore would consist of nothing but gut contents at a body mass of approximately 670 kg.

Discussion

The findings of this study suggest while there appear to be no relevant differences in the allometry of the liver mass and the mass of the gastrointestinal contents, differences do exist between mammals, birds, and reptiles with respect to the allometry of heart, kidney, and the gastrointestinal tissue mass. When compared to allometric equations found by Else and Hulbert (1985) for reptiles, the animals in our study generally achieved higher organ weights for their body masses.

Given the variety of mammal, bird, and reptile species, and the limited selection of species available for the derivation of allometric equations, such results need to be considered with caution. Organ masses in reptiles as well as other clades can be influenced by sex, reproductive status and hibernation status (Telford Jr. 1970; Beuchat & Braun 1988) or food availability and

quality (Relyea & Auld 2004; Naya et al. 2005; Naya & Bozinovic 2006). However, in the collection of allometric equations of Calder (1996) which was used as reference here, there is no evident separation of data for such factors; therefore, the undifferentiated inclusion of data appeared justified for a comparison between clades here.

In correspondence with expectations linked to the differences in metabolism, with low metabolic rates in reptiles and higher rates in birds as compared to mammals (McNab 2002), the organ masses for heart and kidney showed higher values for *a* in the same sequence (Table 1). Similarly, birds exceed mammals in the capacity and the weight of their respiratory system (Lasiewski & Calder 1971; Calder 1996; Maina 2006), but lung masses of mammals and reptiles are similar at similar body masses (Else & Hulbert 1985).The most impressive difference in organ mass between reptiles on the one hand, and mammals and birds on the other, is in the tissue of the gastrointestinal tract. Whereas the contents of the gastrointestinal tract appear to be similar in herbivorous mammals, reptiles and birds (Parra 1978; Bjorndal 1997), the endothermic clades have significantly higher gut tissue masses. Although intestinal microvilli area does probably not differ significantly between herbivorous reptiles and mammals (Ferraris et al. 1989), there is a significant difference in the intestinal surface area between the two clades, mainly due to differences in intestinal length (Karasov et al. 1985; Karasov & Diamand 1985; Karasov et al. 1986; Ferraris et al. 1989). Birds and mammals have distinctively longer small intestines than reptiles (Stevens & Hume 1995), and in birds, the muscular gizzard additionally increases gut tissue mass.

The choice of the allometric equation for the extrapolation of organ tissue masses thus can have relevance for the outcome of organismal reconstructions (Table 2). Using organ allometries for ectothermic organisms (reptiles) should yield generally lower estimates. However, when extrapolating to gigantic body masses by the use of allometric equations such as those derived in the present study, a conceptual problem arises (Table 2). Any slight differences in the allometric exponent *b* will, at very large body masses, lead to very different results, which may, in their scope and ranking, even be different from the observed ranking (see Table 1) based on *a*. In Table 2, it can be seen that when the exact equations from Table 1 are used for the estimation of organ masses in a 38 ton dinosaur in the "allometric approach", the derived reptile equation would lead to dramatically higher estimates for the liver mass, although reptiles would be assumed to have similar (this study) or even slightly lower liver masses than mammals (Else & Hulbert 1985). This paradoxical result is caused by the difference in the allometric exponent *b* (1.061 in reptiles as opposed to 0.87 in mammals). Evidently, at extrapolations to such gigantic masses, the error in the estimation of *b* inherent in the use of imperfect datasets is too large to yield realistic results. A potential solution to overcome this effect, especially when comparing different sets of calculations, is to assume a common exponent *b* for all clades. In our case, where the 95% confidence interval for *b* always included 1.0 (linearity) in the reptiles, we suggest that in the absence of information on 95% confidence intervals in birds and mammals, all correlations can be assumed to be linear. This approach leads to a consistent ranking of extrapolated organ masses according to the reptile-mammal-bird sequence that can be observed in the original equations (Table 1).

Whether we assume that a reptile (ectotherm) or mammal/bird (endotherm) equation should be used for a 38 ton-sauropod dinosaur can lead to a difference in estimated gut tissue mass of more than 1670 kg (or 4.4 % of the assumed body mass). In the case of sauropods, it has been postulated that these animals underwent an ontogenetic shift in their metabolic rate, from juvenile endotherms to adult mass-homoetherms (with low metabolic rates) (Farlow 1990; Sander & Clauss 2008), and intestinal length is usually considered to reflect metabolic rate (Williams et al. 2001). This view of sauropod metabolism would, for example, imply, due to the apparent association of intestinal length and metabolism, that the growth of intestinal tissue mass was less during ontogeny in sauropods than it is in mammals. This view would therefore justify the use of "reptile equations" for adult sauropods, thus alleviating theoretical constraints on the capacity of the coelomic cavity. Gunga et al. (2008) had already concluded that the coelomic cavity of a 38 ton-sauropod dinosaur (*Brachiosaurus brancai*), which they assumed to harbour a volme of 32 m^3 according to their body size reconstructions, provided more space than necessary for most of the organs of this cavity (including a proportion of the skeleton, the blood volume, and the muscle mass, but without accounting for mesenteries, coelomic fat, and reproductive organs), which they estimated at 21 m^3. Using our "linear" approach and the reptile functions (Table 2), and adopting a linear approach based on the mammal functions used by Gunga et al. (2008) for those organs which we could not include in our study, we arrive at a volume estimate of only 17.6 m^3. Evidently, even when considering that mesenteries, fat, and reproductive organs are not included in these calculations, the current data allows for a dramatic increase in organ masses in the reconstruction of sauropod dinosaurs. As sauropods are thought to have herterogenous (avian-type) lungs with an airsac system (Sander & Clauss 2008), a part of the space in the coelomic cavity was probably filled with these airsacs. In birds, the lungs and

airsacs may account for as much as 20% of the total body volume (King 1966): in the 38 ton-sauropod of Gunga et al. (2008), with an estimated total volume of approximately 47.6 m^3, this would represent a total lung and aisac volume of 9.5 m^3. Even if we assume that the majority of this volume was placed within the coelomic cavity, the reconstruction would still allow for theoretical increases in any organ masses.

Given that we must assume elevated metabolic rates in certain ontogenetic stages, and no mastication of ingesta (Farlow 1990; Sander & Clauss 2008), the gastrointestinal contents could be a plausible candidate for a mass above estimates based on regressions from extant animals – to allow a thorough digestion in spite of absent food comminution and without compromising intake (Farlow 1987; Clauss et al. 2007b). In order to roughly estimate whether gut capacity should be considered a limiting factor in sauropods, we extrapolated the dry matter intake for sauropods from Hummel et al. (2008) to a 38 ton-sauropod; these values are given at four assumed levels of metabolism. Assumptions were made for a medium-quality and a low-quality diet (with presumed apparent dry matter and energy digestibilities of 44 and 33 %, respectively); additionally, we estimated the dry matter concentration in sauropod gut contents to be 15 %, a level similar to that of mammals (but probably lower than in reptiles, M. Clauss, pers. obs.). Using the equation by Holleman & White (1989) that links dry matter intake, digestibility, dry matter gut capacity, and ingesta retention time, we can estimate the mean retention time in hypothetical sauropods of varying metabolic level (Table 3; see electronic appendix for details). At the normal, extrapolated gut capacity, retention times are between 4 and 8 days for a medium-quality food; a doubling of the gut content – which would still leave about 10m^3 of the presumed coelomic cavity unoccupied for mesenteries, fat, and reproductive organs – would result in

retention times between 8 and 16 days. Thus, estimated retention times fall within the range of 11 days measured in Galapagos tortoises (*Geochelone nigra*) (Hatt et al. 2002), which – as extant reptiles – do not chew their food.

In conclusion, this study as well as that of Gunga et al. (2008) show that, from the aspect of organismal reconstruction based on body volume and organ estimates, no restrictions are evident in the sauropod *bauplan*; on the contrary, given our current equations for organ allometry, the body cavity of sauropods as it reconstructed allows leeway for any adjustments in organ size that one might deem necessary to fit their – potentially unique – lifestyle. In particular, digestive physiology is an unlikely candidate for a potential body size limitation in sauropods.

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Table 1: Statistics of regression analysis according to the equation organ mass = a BM^b (masses in kg) for reptiles. Allometric organ equations for birds and mammals are from Calder (1996); data for gut contents of mammals from Clauss et al. (2007a) and for birds from Herd & Dawson (1984), Dawson et al. (1989) and Grajal (1995).

Organ	**Clade (species)**	**BM range (kg)**	**a**	**95% CI**	**b**	**95% CI**	**R^2**	**P**
Heart	Reptile (28)	0.008-1.052	0.005	0.0036-0.0070	1.055	0.929-1.181	0.919	>0.001
	Mammal (568)	-	0.006	-	0.98	-	-	-
	Bird (n.a.)	-	0.009	-	0.94	-	-	-
Kidney	Reptile (28)	0.008-0.990	0.006	0.0037-0.0085	0.945	0.792-1.099	0.860	>0.001
	Mammal (138)	-	0.007	-	0.85	-	-	-
	Bird (334)	-	0.009	-	0.91	-	-	-
Liver	Reptile (29)	0.008-0.715	0.033	0.0219-0.0484	1.066	0.917-1.216	0.888	>0.001
	Mammal (175)	-	0.033	-	0.87	-	-	-
	Bird (n.a.)	-	0.033	-	0.88	-	-	-
GIT	Reptile (29)	0.008-1.123	0.031	0.0207-0.0458	1.159	0.997-1.321	0.889	>0.001
	Mammal (41)	-	0.075	-	0.94	-	-	-
	Bird (n.a.)	-	0.090	-	0.99	-	-	-
GIT wet contents	Reptile (12)	0.059-3.150	0.080	0.0584-0.1104	1.389	1.195-1.583	0.962	>0.001
	Mammal (74)	0.015-3140	0.107	0.094-0.121	1.062	1.029-1.095	0.983	>0.001
	Bird (3)	0.712-35.330	0.044	0.000-545.1	1.204	-3.347-5.755	0.919	0.184

n.a. = not available

Table 2: Extrapolation of organ masses (in kg) of a hypothetical 38000 kg vertebrate (the estimated mass of *Brachiosaurus*, a sauropod dinosaur, Gunga et al. 2008) under different assumptions: "linear approach" = assuming linear scaling with body mass for all clades, i.e. b = 1.0, using values for a from Table 1; "allometric approach" = using the exact equations as given in Table 1. Note that due to small differences in the exponent b, extrapolations using the exact equations will yield fundamentally different results.

	------- linear approach -------			**----- allometric approach -----**		
	Reptile	Mammal	Bird	Reptile	Mammal	Bird
Heart	190	228	342	339	185	182
Kidney	228	266	342	128	55	132
Liver	1254	1254	1254	2515	318	354
GIT tissue	1178	2850	3420	6300	1514	3078

Table 3: Estimation of ingesta mean retention time (MRT) in a hypothetical 38000 kg vertebrate (the estimated mass of *Brachiosaurus*, a sauropod dinosaur, Gunga et al. 2008) at different levels of metabolism and hence daily food intake (for 'medium' and 'low' quality food, Hummel et al. 2008) at the extrapolated gut capacity of 610 kg dry matter (from Table 1, linear approach, assuming a dry matter concentration of 15 % in gut contents) and at a doubled gut capacity; MRT estimated according to Holleman & White (1989). DMI = dry matter intake; DFE = dry faecal excretion.

Level of metabolism	**DMI (kg/d)**	**DFE (kg/d)**	**MRT hours (days)**	
			Gut capacity	
			610 kg DM	***1220 kg DM***
Medium quality food				
Reptile	20	11	927 (39)	1854 (77)
Intermediate 1	96	53	197 (8)	394 (16)
Intermediate 2	140	78	135 (6)	269 (11)
Mammal	188	104	100 (4)	201 (8)
Low quality food				
Reptile	28	18	639 (27)	1278 (53)
Intermediate 1	127	84	139 (6)	278 (12)
Intermediate 2	186	124	94 (4)	189 (8)
Mammal	250	166	70 (3)	141 (6)

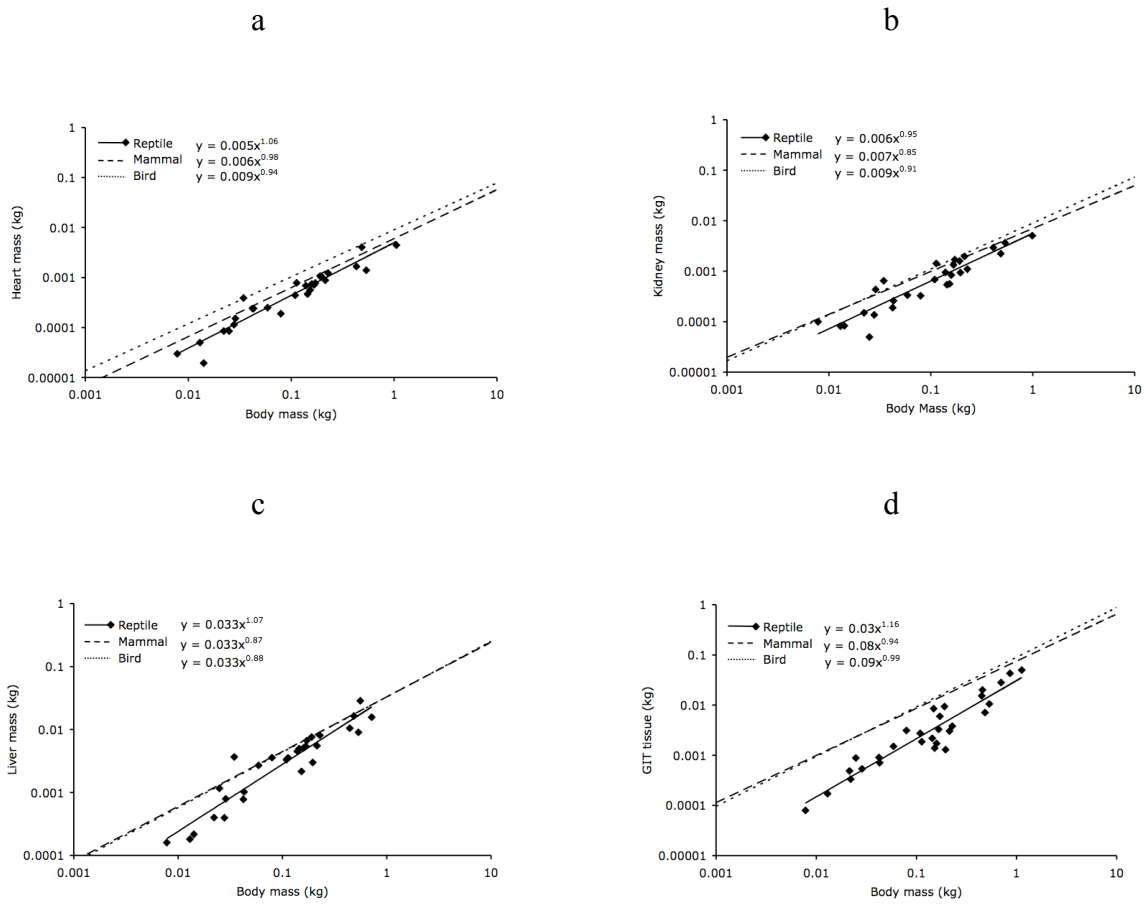

Fig. 1. Correlations of body mass and organ mass in reptiles (diamonds, solid line), mammals (interrupted line) and birds (dotted line) for a) heart, b) kidneys, c) liver and d) gastrointestinal tissue. Reptile data from this study (see electronic appendix), mammal and bird regression lines from Calder (1996).

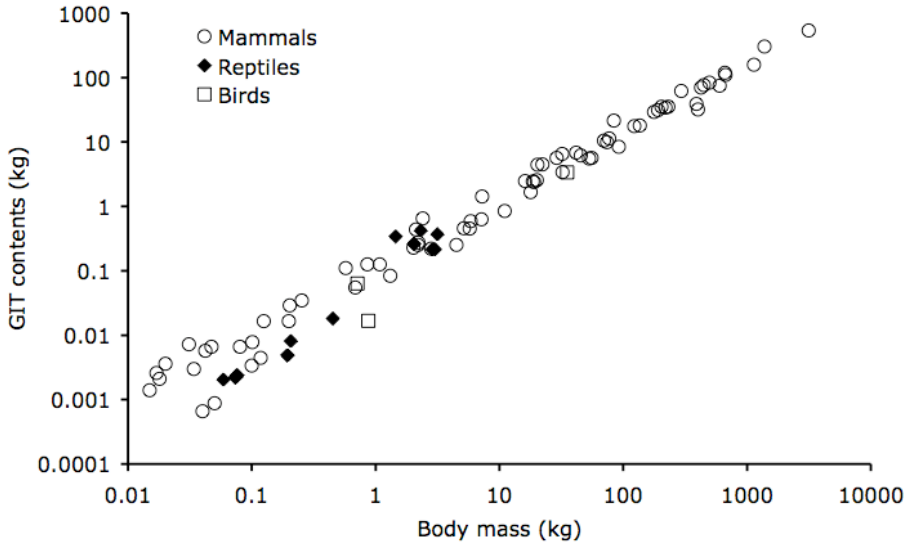

Fig. 2. Wet contents mass of the total gastrointestinal tract in mammals (data from Clauss et al. 2007a), birds (data from Herd & Dawson 1984, Dawson et al. 1989, Grajal 1995) and reptiles (data in electronic appendix) in relation to body mass.

Chapter 2

Herbivorous reptiles and body mass: effects on food intake, digesta retention, digestibility and gut capacity, and a comparison with mammals

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Herbivorous reptiles and body mass: effects on food intake, digesta retention, digestibility and gut capacity, and a comparison with mammals

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Abstract

Differences in the allometric scaling between gut capacity (with body mass, $BM^{1.00}$) and food intake (with $BM^{0.75}$) should theoretically result in a scaling of digesta retention time with $BM^{0.25}$ and therefore a higher digestive efficiency in larger herbivores. This concept is an important part of the so-called 'Jarman-Bell principle' (JBP) that explains niche differentiation along a body size axis in terms of digestive physiology. Empirical data in herbivorous mammals, however, do not confirm the scaling of retention time, or of digestive efficiency, with body mass. Here, we test these concepts in herbivorous reptiles, adding data of an experiment that measured food intake, digesta retention, digestibility and gut capacity in 23 tortoises (*Testudo graeca*, *T. hermanni* , *Geochelone nigra*, *G. sulcata*, *Dipsochelys dussumieri*) across a large BM range (0.5-180 kg) to a literature data collection. While dry matter gut fill scaled to $BM^{1.07}$ and dry matter intake to $BM^{0.76}$, digesta mean retention time (MRT) scaled to $BM^{0.17}$ for species > 1 kg. Food intake level was a major determinant of MRT across reptiles and mammals. In contrast to dietary fibre level, BM was not a significant contributor to dry matter digestibility in a General Linear Model. Digestibility coefficients in reptiles depended on diet nutrient composition in a similar way as described in mammals. Although food intake is generally lower, and digesta retention longer, in reptiles than in mammals, digestive functions scale in a similar way in both clades, indicating homology of digestive physiology. The reasons why the theoretically derived JBP has little empirical support remain to be investigated. Until then, the JBP should not be evoked to explain niche differentiation along a body size axis in terms of digestive physiology.

Introduction

Body size is often considered the most important characteristic of an organism (Peters 1983; Calder 1996). Small or large body size may have effects that convey comparative advantages and hence favour evolution of certain body sizes (Hone and Benton 2005). Studying the correlation of body size with physiological functions allows us to not only extrapolate measurements to species of known body size that have not been investigated yet, but also to compare species across the body size range (Karasov and Martínez del Rio 2007). Relationships between a physiological measure and body size (mostly measured as body mass, BM) are often not linear (isometric) but follow 'another pattern', or, in other words, an allometric pattern (Peters 1983).

Allometric considerations play an important role in theoretical concepts about niche differentiation in mammalian herbivores (reviewed in Clauss et al. 2007a). In brief, the so-called Jarman-Bell principle (JBP) (Bell 1971; Geist 1974; Jarman 1974) explains the observation that herbivores of larger size ingest food of lower nutritional quality with the putatively increasing gut capacity per unit food intake in larger organisms. As gut capacity scales to $BM^{1.00}$, but energy requirements and food intake to $BM^{0.75}$, larger animals have theoretically more gut capacity available per unit food intake, which translates into longer digesta retention times that should scale to $BM^{0.25}$ (Parra 1978; Demment and Van Soest 1985; Illius and Gordon 1992). According to this concept, larger animals should achieve higher digestibilities (on similar foods) due to their longer digesta retention times. However, although this concept has found widespread distribution, empirical evidence does neither indicate a systematic scaling of digesta retention, nor an increase in digestibilities with body mass (Smith 1995; Pérez-Barberìa et al. 2004; Clauss et al. 2007a; Pérez-Barberìa et al. 2008; Clauss et al. 2009; Steuer 2010).

The theoretical approach of the JBP is not related to a particular level of metabolism. Therefore, the same considerations should apply to other groups of vertebrate herbivores – for example reptiles (Parmenter 1981). The microbial digestion of plant cell wall in reptiles has many similarities to that in herbivorous mammals (Troyer 1991; Bjorndal 1997). In herbivorous reptiles, limited evidence suggests that gut capacity scales to BM in a similar, linear fashion as it does in mammals (Troyer 1984a; Bjorndal 1997; Franz et al. 2009). Energy requirements – estimated as basal metabolic rates or field metabolic rates – scale roughly to metabolic body mass ($\sim BM^{0.75}$) as they do for mammals (Bennett and Dawson 1976; Nagy et al. 1999). Therefore, it is reasonable to assume that food intake scales in a similar fashion. Intake has so far only been analysed across a larger body size range within species, with conflicting results: Meienberger et al. (1993) found that dry matter intake (DMI) scaled to $BM^{0.71}$ in desert tortoises (*Xerobates agassizii*), and the results from Hamilton and Coe (1982) in Aldabra tortoises (*Dipsochelys dussumieri*) translate into a scaling of DMI with $BM^{0.77}$-$BM^{0.81}$, whereas Bear et al. (1997) found a linear scaling of DMI with BM in growing green iguanas (*Iguana iguana*). There is also no uniformity in the results reported for relationships between BM and digesta retention or digestibility. Several authors showed that within species, digesta retention was hardly correlated to BM or other measures of body size (Parmenter 1981; Bjorndal 1987; 1989; Brand et al. 1990; Meienberger et al. 1993; Hatt et al. 2002), whereas only two studies demonstrated an increase of digesta retention with BM in reptiles (Hamilton and Coe 1982; Troyer 1984b). Instead, some authors suggested that the level of food intake determined digesta retention (Bjorndal 1987; 1989; Zimmerman and Tracy 1989; Brand et al. 1990; van Marken Lichtenbelt 1992; Meienberger et al. 1993); the same conclusion has been reached for mammals, both within and between species (Clauss et al. 2007a; 2007b; 2008). Except for one study in green turtles

(*Chelonia mydas*) (Bjorndal 1980), no effect of BM on digestibility was found in herbivorous reptiles so far (Hamilton and Coe 1982; Troyer 1984b). Although in part contradictory, these findings suggest that herbivorous reptiles might show a similar pattern as herbivorous mammals: a scaling of gut capacity and food intake as predicted by the JBP, without the theoretically corresponding scaling of digesta retention and digestive efficiency.

In order to test this assumption, we performed intake, passage and digestion studies with herbivorous tortoises of five species across a BM range from 0.5-180 kg, and added these data to a data collection on digestive parameters in herbivorous reptiles from the literature.

Materials and Methods

We performed intake and respiration chamber measurements in 23 individual tortoises of the species Testudo graeca (n=4), T. hermanni (n=6), Geochelone nigra (n=2), G. sulcata (n=8), and Dipsochelys dussumieri (n=3). Animals were kept individually for 30 days at 27–30°C for intake measurements after an adaptation period of one week. The diet consisted of grass hay (whole or chopped in varying degrees) and salad in varying proportions. Water was available ad libitum at all times. Faeces were collected from the enclosure floor, which consisted of plastic in the case of smaller tortoises, plastic, wood panels or concrete in the case of mid-sized tortoises, and the natural floor of the Masoala Exhibit at Zurich Zoo (Bauert et al. 2007) in case of the three largest individuals. While loss of faecal material or contamination of faeces was not judged substantial in individuals from 5 kg upwards, smear losses of faeces (from animals moving over their own faeces) in the smallest tortoises was judged problematic. Food offered and left over was quantified, and faeces were collected completely on a daily basis. If several defecations occurred in one day, they were sampled individually. Representative subsamples were used to determine

dry matter (DM), ash, crude protein, neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentrations using standard methods (AOAC 1997). Daily DM intake (DMI) was quantified for the whole trial period. Additionally, we used the DMI and the faecal excretion data of those days that were separated by the resulting particle mean retention time (MRT, see below) for each individual for the calculation of digestibility. Apparent digestibility (aD) of nutrients and energy were calculated as

$$(\text{Intake} - \text{excretion}) / \text{intake} \times 100.$$

MRT was determined by feeding a particle (chromium-mordanted fibre, < 2 mm) and a solute (cobalt-EDTA) marker prepared according to Udén et al. (1980). The solute marker was only given to animals > 2 kg. Marker analysis followed the procedure outlined by Behrend et al. (2004) and Hummel et al. (2005); in doing so, wet ashing with sulphuric acid was followed by atom absorption spectroscopy. The MRT of the total gastrointestinal tract was calculated according to Thielemanns et al. (1978) as

$$MRT = \sum(t_i \times dt \times c_i) / \sum(dt \times c_i)$$

with t_i = time after marker application (h), dt = time interval represented by marker concentration (calculated as $(((t_{i+1} - t_i) + (t_i - t_{i-1})) / 2)$, and c_i = faecal marker concentration at time i (mg/kg DM). The marker was assumed to have been excreted completely once the faecal Co and Cr concentrations were similar as prior to marker application. The selectivity factor was calculated as $MRT_{particles}/MRT_{solutes}$. We followed Barboza (1995a) in calculating the indigestible gut content (V_N) and the total gut content (V) according to Holleman and White (1989) as

$$V_N = F * MRT$$

with F = faeces output (kg DM/h) and MRT = the average (2 mm) particle passage time through the entire digestive tract (h), and

$$V = (V_N - (V_N /(1 – (aD\ DM/100)))/\ln(1 – (aD\ DM/100))$$

assuming an exponential absorption of ingested food with time spent in the digestive tract.

Comparative data were compiled from the literature (Bjorndal 1980; Karasov et al. 1986; Nagy and Medica 1986; Bjorndal 1987; 1989; Davenport et al. 1992; van Marken Lichtenbelt 1992; Bjorndal and Bolten 1993; Meienberger et al. 1993; Barboza 1995a; b; Baer et al. 1997; Hailey 1997; Liesegang et al. 2001; Hatt et al. 2002; Hatt et al. 2005; Bouchard and Bjorndal 2006). Publications that did not allow linking body mass data to other measurements were not included.

Data were analysed by correlation analysis (after ln-transformation of parameters without normal distribution), by General Linear Models (GLM; assessing normal distribution of studentized residuals), and using regression analysis indicating 95% confidence intervals (*95%CI*) according to $y = a\ BM^b$ (after ln-transformation) or $y = ax + b$ (without transformation). Analyses were performed with PSAW 18.0 (SPSS Inc., Chicago, IL). In cases where data on MRT and transit time (TT, time of first marker appearance) from the literature were combined, we speak of 'passage times'.

Results

Own results

The results of our own experiments are summarized in Table 1. DMI ($g\ d^{-1}$) scaled to 4.8 (*95%CI* 3.6-6.5) $BM^{0.75\ (95\%CI\ 0.64\text{-}0.87)}$ (n=22, r^2=0.90, p<0.001) for intake measured during the whole trial period, and to 4.8 (*95%CI* 3.7-6.3) $BM^{0.80\ (95\%CI\ 0.70\text{-}0.90)}$ (n=22, r^2=0.93, p<0.001) for intake during those days used for digestibility calculation.

BM was positively correlated to the NDF (n=22, R=0.93, p<0.001) and ADF (n=21, R=0.45, p=0.040) of the ingested diet. BM, the relative DMI (rDMI, g^{-1} $kg^{-0.75}$ d^{-1}) and diet NDF content were all negatively correlated to digestibility estimates (e.g. for aD DM n=22, R=-0.83, p<0.001; n=22, R=-0.48, p<0.025; and n=22, R=-0.83, p<0.001; or for aD NDF n=21, R=-0.69, p=0.001; n=21, R=-0.58, p=0.006; and n=21, R=-0.66, p=0.001, respectively). rDMI was not correlated to diet NDF content (n=22, R=0.26, p=0.236). In a GLM with aD DM as the dependent variable and BM, rDMI and diet NDF content as covariates (n=22, r^2=0.78, F=20.850, p<0.001), only NDF (F=15.595, p=0.001) and rDMI (F=5.862, p=0.026) were significant but not BM (F=1.111, p=0.297). The regression equation for the relationship of aD of organic matter (OM) and NDF was aD OM = 125.7 (*95%CI* 106.2-145.2) – 1.02 (95%CI *-1.40 - -0.64*) NDF (n=19, r^2=0.65, p<0.001).

The marker excretion curves showed single marker excretion peaks (Fig. 1a,c,d) in 16 animals and double marker peaks (Fig. 1b) in 6 cases. In one animal, the marker excretion pattern and faecal marker concentration indicated that the majority of the marker had not been excreted within the experimental period. Because this animal (*G. sulcata* 16) also had the lowest rDMI (1.35 g^{-1} $kg^{-0.75}$ d^{-1}) of all animals, this interpretation was considered plausible, and MRTs were not calculated for this animal. A gradual marker increase prior to the peak (Fig. 1c) was observed in six cases; a gradual particle marker decrease after the peak (Fig. 1d) was only observed in two cases.

$MRT_{particles}$ (h) scaled to 145 (*95%CI* 113-186) $BM^{0.16\ (95\%CI\ 0.06\text{-}0.25)}$ (n=22, r^2=0.37, p=0.003). If only animals > 2 kg were considered, there was no significant scaling for $MRT_{particles}$ ($BM^{0.04\ (95\%CI\ -0.20\text{-}0.28)}$, n=12, r^2=0.01, p=0.726) and $MRT_{solutes}$ ($BM^{-0.03\ (95\%CI\ -0.71\text{-}0.66)}$, n=12, r^2=0.00, p=0.935). rDMI was not correlated to $MRT_{particles}$ (n=21, R=-0.24, p=0.298), but diet NDF was

(n=21, R=0.46, p=0.037). In a GLM with $MRT_{particles}$ as the dependent variable and BM, rDMI and diet NDF content as covariates (n=21, r^2=0.47, F=5.082, p=0.011), only NDF (F=12.215, p=0.003) and rDMI (F=6.402, p=0.022) were significant but not BM (F=2.917, p=0.106).

Gut fill (kg DM) scaled to 0.013 (*95%CI* 0.009-0.017) $BM^{1.07\ (95\%CI\ 0.95\text{-}1.19)}$ (n=21, r^2=0.95, p<0.001).

Complete data collection

We compared both, available individual data for DMI and calculated species means, to the collection of species means for herbivorous mammals from Clauss et al. (2007a). Because of an overrepresentation of small individuals with low food intake in the total dataset, the scaling exponent of all individuals was higher than $BM^{0.75}$ (Fig. 2a). In contrast, species means scaled to metabolic body mass (Fig. 2b). The *95%CI* for the factor *a* did not overlap between mammals and reptiles; this factor was ten times lower in reptiles than in mammals.

In the literature, both MRT and transit times (TT) are recorded for reptiles. Given the predominance of abrupt, single-peak excretion patterns in our own data (Fig. 1a), one could assume that TT should be representative for MRT in reptiles (Bjorndal 1997); alternatively, one could assume that TT are usually shorter than MRT. If all individual MRT and TT data are combined, there is no scaling of passage time with BM in reptiles (Fig. 3a). Using species averages (if both TT and MRT were given for a species, only MRT data were used), MRT scaled to $BM^{0.17}$ (Fig. 3b). If the BM range was confined to species > 1 kg (similar to the considerations in Clauss et al. 2007a), the scaling exponent was similar $BM^{0.17\ (95\%CI\ -0.05\text{-}0.38)}$ but no longer significant (n=11, r^2=0.26, p=0.112). When compared for similar BM, reptile passage times were on average five times longer than mammal MRTs.

Using all individual MRT and TT data in a GLM with BM and rDMI as covariates (n=70, r^2=0.35, F=18.377, p<0.001), only rDMI (F=36.496, p<0.001) was significant but not BM (F=0.239, p=0.627). If only MRT was used as the dependent variable in the GLM (n=30, r^2=0.08, F=1.177, p=0.324), neither rDMI nor BM were significant. Using species' average MRT and TT data in a GLM with BM and rDMI as covariates (n=17, r^2=0.10, F=0.762, p=0.485), neither rDMI nor BM were significant. If only species-average MRT data were used as the dependent variable in the GLM (n=10, r^2=0.10, F=0.379, p=0.698), again neither rDMI nor BM were significant.

A comparison of the relationship between rDMI and passage parameters between mammals (species averages) and reptiles (individual data for MRT and TT) indicated a common pattern of increasing passage time with decreasing intake (Fig. 4). At similar intake levels reptiles still had about 1.6 times longer passage times; the difference was, however, not significant due to overlapping confidence intervals (Fig. 4).

Comparing the calculated DM gut fill of the tortoises of this study with similar data for mammals shows that in both groups, gut fill scales linearly with BM (Fig. 5).

A combination of literature and own data on the relationship showed a decrease of DM digestibility with dietary NDF content (Fig. 6a) that only tended towards significance (n=45, R=-0.26, p=0.086). When using data on dietary ADF content and the digestibility of organic matter, the negative correlation was significant (n=38, R=-0.43, p=0.007). In a GLM with organic matter digestibility as the dependent variable and BM, rDMI and dietary ADF as covariates (n=38, r^2=0.21, F=2.950, p=0.046), only ADF (F=5.402, p=0.026) was significant but not BM (F=0.217, p=0.644) or rDMI (F=0.693, p=0.411). BM was not correlated to the digestibility of NDF in the overall dataset (n=48, R=-0.029, p=0.847; Fig. 6b). The regression equation for the

relationship of aD of organic matter (OM) and NDF was aD OM = 87.7 (*95%CI* 65.6-109.8) – 0.47 (95%CI -*0.94 - 0.00*) NDF (n=35, r^2=0.11, p=0.051).

Relating data on dietary crude protein content to the content of the digestible crude protein content (Fig. 7) allows estimation of the true digestibility and endogenous/metabolic losses (Robbins 1993). Estimated true protein digestibility was 81% for the whole dataset, with endogenous protein losses estimated at 2.49 g/100 g DMI.

Discussion

This study confirms that herbivorous reptiles have a lower food intake and longer digesta retention times than herbivorous mammals, whereas gut capacity is comparable. Additionally, Fritz at al. (2010) showed that reptiles have larger digesta particles than mammals . These findings corroborate the assumption that a higher metabolic level (as in mammals) is linked to a higher food intake (Karasov et al. 1986). Because of the similarity in anatomy (an 'amniote *bauplan*'), gut capacity remains more or less constant and hence higher food intake leads to shorter digesta retention, which could compromise digestibility (Meienberger et al. 1993; Clauss et al. 2007b). Therefore, adaptations for particle size reduction become crucial for the evolution of a higher level of metabolism, because a reduction in particle size can compensate for shorter digesta retention (Bjorndal et al. 1990; Clauss et al. 2009; Schwarm et al. 2009).

A fascinating result of the comparisons between herbivorous mammals and reptiles is that although differences in the levels of various physiological measures are found, the scaling of these measures with BM is similar between both groups (Fig. 2-5; cf. Fig. 1 in Fritz et al. 2010), suggesting fundamental scaling principles for terrestrial vertebrate herbivores.

Limitations of this study

One limitation of this study could have been the smear losses of faecal material in the smallest individuals mentioned in the method section. Although this will not have influenced intake and passage measurements, these putative smear losses could have led to particularly high calculated digestibilities in the smallest individuals (Table 1) and thus led to a steeper NDF-aD DM-relationship in the data from this study as compared to literature data (Fig. 6a). However, digestibility coefficients of similar (high) magnitudes had also been observed in larger tortoises where smear losses should not be a problem (Liesegang et al. 2001).

A period of 30 days for the intake and digestion studies was adequate in all but one case for passage marker recovery. However, a longer time period would be desirable for the determination of intake, faecal excretion and hence digestibility.

A major difficulty in this study was the variation of nutrient composition in the ingested diet. Because recording voluntary food intake and corresponding passage measurements was our defined aim, and thus force-feeding of animals (with a uniform diet) was not an option, diet selection on the part of the animals could not be prevented. Actually, hay offered to larger tortoises would physically not have been acceptable for the smallest individuals. The increase of dietary NDF with BM reflects the opportunity for selective feeding in smaller individuals already noted by Bjorndal and Bolten (1992). Therefore, effects of body size on digestibility, need to be assessed with the difference in the ingested diet in mind (i.e. including nutrient levels in a General Linear Model).

Passage marker excretion in reptiles

Rick and Bowman (1961) already noted that digesta passage in tortoises was very long, exceeding 14 days in an experiment of seed passage in two *Geochelone nigra* specimens (5 and 11 kg). Besides the generally much longer retention time, the excretion of passage markers also differs in its pattern between reptiles and mammals. In many hindgut-fermenting mammals, such as horses, tapirs, rhinoceroses or elephants, the excretion pattern of the marker is usually that of a peak with a steep increase and a gradual decline (Udén et al. 1982; Loehlein et al. 2003; Clauss et al. 2010; Steuer et al. 2010), indicative of a mixing compartment (Martinez del Rio 1994; Jumars 2000). However, such a pattern is only rarely found in reptiles (Fig. 1d; cf. Zimmerman and Tracy 1989; Barboza 1995a) and a steep-peaked pattern with an abrupt decline is the more common finding (Fig. 1a; cf. Karasov et al. 1986; Hatt et al. 2002). Such a pattern indicates a low degree of digesta mixing and a passage of digesta as a plug in a plug-flow reactor and matches the generally tubiform shape of the reptilian digestive tract (with the exception of colonic compartimentalisation in iguanids) (Bjorndal 1997). The pattern of an even more gradual increase in passage marker before the peak than the subsequent decrease observed in this and other studies (Fig. 2c; cf. Barboza 1995a; Hatt et al. 2002) could be a consequence of using particle markers that are smaller than the average digesta (note the higher particle size of ingesta in reptiles as described by Fritz et al. 2010), and partly washed out of their plug in the gastrointestinal tract by the fluid fraction. Although the absolute duration of digesta passage is much higher in reptiles than in mammalian herbivores, $MRT_{particles}$ is longer than $MRT_{solutes}$ by a factor of only 1.4-2.6 in this study, 1.9-2.1 in the study of Barboza (1995a), and 0.8-1.5 in the study of Hatt et al. (2002), and thus in a similar range as that observed in mammals (Clauss et al. 2010; Steuer et al. 2010). This indicates that relative to food intake and digesta passage, reptiles

secrete similar amounts of fluids into their digestive tract, and thus submit digesta to a similar degree of 'washing' as mammalian herbivores.

Digestion in reptiles

Bjorndal (1997) summarized physiological data that indicates that herbivorous reptiles achieve similar digestibilities as mammalian herbivores. The results of our study support this conclusion. Digestibility is usually a negative function of dietary fibre content (Karasov and Martínez del Rio 2007), as demonstrated within iguanas by Van Marken Lichtenbelt (van Marken Lichtenbelt 1992). The general similarity of this relationship in reptiles with those found in herbivorous mammals is striking (Table 2) and supports previous suggestions that fibre level might influence digestibility in a similar way in both clades (Hatt et al. 2005). Similarly, endogenous protein losses and true protein digestibility, as estimated by regression analysis, are similar between reptiles and mammals (Table 3). This similarity suggests a homology in the fundamental mechanisms of digestion, even if different characteristics of digestive physiology (retention time, temperature constancy, fermentation rate) apply in the different clades.

Body mass

The findings of this study corroborate findings in herbivorous mammals, in which a discrepancy in the scaling of gut capacity, food intake, and digesta retention was documented (Clauss et al. 2007a). In reptiles, as in mammals, the scaling exponent for the relationship of digesta retention and body mass is lower than expected on theoretical terms by the Jarman-Bell principle, and may becomes even smaller when only a body mass range > 1 kg is analysed. The reasons for this absence in scaling remain to be investigated. The level of food intake is a major determinant of

digesta retention both within and between species, which emphasizes that variation in the metabolic level between species may be more important for digesta retention than their body mass. Additionally, differences between species in their particular digestive niches – such as the degree of herbivory or the botanical group of plants they specialize on – are important modulators of digestive adaptations, as shown for browsing and grazing ruminants (Pérez-Barberìa et al. 2004), or for tortoises varying in their intestinal morphology according to their feeding style (Hailey 1997). These results therefore emphasize that even though the theoretical background of the Jarman-Bell principle is appealing, it should not be evoked to explain niche differentiation along a body mass axis in terms of digestive physiology.

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Table 1. Tortoises used in this study, body mass (BM), dry matter intake (DMI), diet nutrient composition and digestibility, mean retention time (MRT) and calculated dry matter gut fill.

Species	ID	BM	DMI		Ingested diet composition				------- Apparent digestibility -------					MRT		Gut fill
			[1])	[2])	Ash	Prot.	NDF	ADF	DM	OM	Prot.	NDF	ADF	part.	sol.	
		kg	g d^{-1}		%DM						%			h		% BM
T. graeca	1	0.52	4.6	5.0	7.3	15.0	37.8	24.5	83					89	-	1.52
T. graeca	2	0.69	3.4	3.6	5.1	11.2	29.6	15.9	85	86	82	69		190	-	1.76
T. graeca	3	0.90	4.2	3.9	6.1	17.0	38.5	19.2	94	94	95	89	88	137	-	0.90
T. graeca	4	0.86	5.9	5.5	7.5	12.8	42.4	28.1	94	95	94	93	92	238	-	2.24
T. hermanni	5	0.91	5.7	5.2	7.1	13.9	42.5	29.2	94	94	93	92	90	90	-	0.78
T. hermann	6	0.96	4.2	4.2	6.0	14.5	34.1	20.9	87	88	87	78	72	143	-	1.10
T. hermann	7	1.01	4.5	4.3	6.1	13.4	39.6	22.1	82		80	74		150	-	1.33
T. hermann	8	1.44	3.9	3.7	5.6	13.8	43.7	22.2	95		95	94	89	133	-	0.48
T. hermann	9	1.64	4.8	4.8	7.0	15.3	33.8	24.3	90	91	91	83	78	96	-	0.46
T. hermann	10	1.72	7.0	6.7	7.6	14.5	44.9	30.2	74	76	76	64	58	147	-	1.35
G. nigra	11	5.30	19.2	18.3	6.0	13.2	47.8	37.8	66	68	71	57	59	197	139	1.82
G. nigra	12	5.70	35.8	35.5	7.6	13.2	51.2	34.4	63	65	65	55	49	131	65	2.18
G. sulcata	13	7.24	35.4	43.2	7.5	12.4	56.3	35.0	51	53	65	44	33	262	152	3.83
G. sulcata	14	10.47	42.7	38.0	6.5	10.9	60.0	40.2	72	74	67	70	70	209	153	2.00
G. sulcata	15	12.23	33.3	32.3	7.9	13.5	55.1	40.7	64	66	72	61	55	368	260	2.63
G. sulcata	16	21.5	20.3	13.5	12.6	13.1	53.7		74	80	78	77	48	-	-	-
G. sulcata	17	26.0	32.1	20.9	10.0	9.5	54.7	30.2	79	81	75	79	72	556	340	1.45
G. sulcata	18	47	103.0	100.8	13.1	10.5	57.8	27.8	62	62	54	62	40	241	99	1.42
G. sulcata	19	48	90.1	90.1	13.4	10.8	56.4	23.8	65	66	53	66	43	266	104	1.28
G. sulcata	20	50	-	-	-	-	-	-	-	-	-	-	-	554	340	-
D. dussumieri	21	104	216.1	175.6	5.8	12.2	63.6	29.7	53	61	75	45	23	210	149	1.28
D. dussumieri	22	140	494.0	342.5	5.5	11.4	66.2	32.1	51	59	71	49	39	203	125	2.13
D. dussumieri	23	180	375.0	283.2	1.3	13.6	64.0	30.0	52	58	69	46	29	202	141	1.24

Prot. crude protein, NDF neutral detergent fibre, ADF acid detergent fibre, DM dry matter, OM organic matter, part. particles, sol. solutes

[1]) DMI used for digestibility calculation (see methods), [2]) DMI during the complete trial period

Table 2. Relationship between the apparent digestibility (aD) of organic matter (OM) in herbivorous reptiles and mammals.

Animal group	equation	Source
Iguana iguana	aD OM = 96 – 1.15 NDF	(van Marken Lichtenbelt 1992)
Tortoises	aD OM = 126 – 1.02 NDF	this study
Herbivorous reptiles	aD OM = 88 – 0.47 NDF	data collection in this study
Browsing rhinoceroses	aD OM = 101 – 0.98 NDF	(Clauss et al. 2006)
Grazing rhinoceroses	aD OM = 81 – 0.42 NDF	(Clauss et al. 2006)

NDF in % dry matter

Table 3. Relationship between the crude protein content (CP, in % dry matter) of the diet and its digestible CP (dCP, in % dry matter) in herbivorous reptiles and mammals. Note that the slope of the equation represents the true protein digestibility, and the intercept the endogenous/metabolic protein losses.

Animal group	equation	Source
Herbivorous reptiles	dCP = 0.81 CP – 2.5	data collection in this study
Horses	dCP = 0.86 CP – 2.8	(collection in Clauss et al. 2006)
Black rhinoceros	dCP = 0.88 CP – 3.7	(Clauss et al. 2006)
Indian rhinoceros	dCP = 0.71 CP – 1.5	(Clauss et al. 2005)
Hippopotamuses	dCP = 0.86 CP – 1.8	(Schwarm et al. 2006)

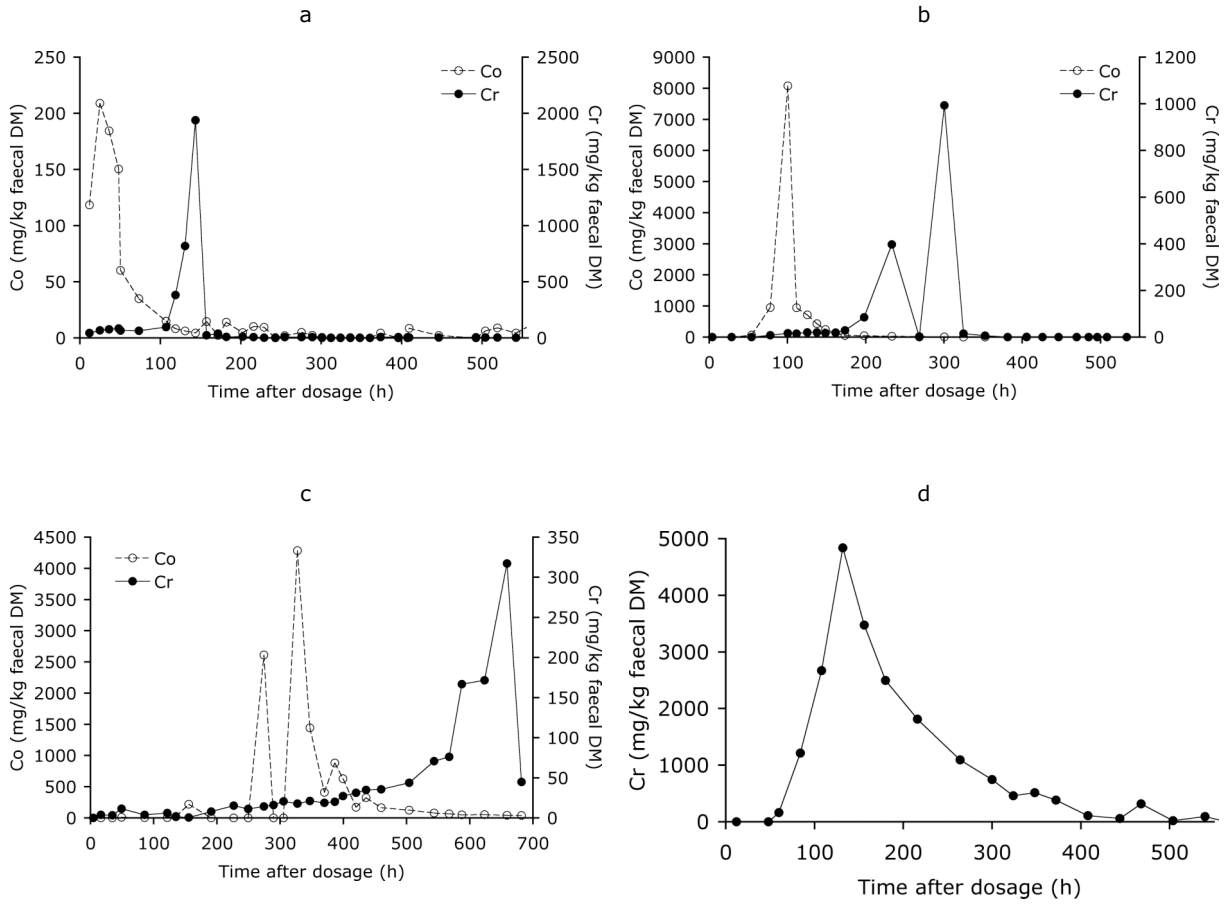

Fig. 1. Marker excretion patterns in herbivorous tortoises: a) single marker peaks (*Geochelone nigra* 12) as seen in 16 animals of this study; b) double particle marker peak (*Geochelone sulcata* 19) as seen in six animals of this study; c) a very gradual increase in particle marker excretion prior to the major excretion peak (*Geochelone sulcata* 20) as seen in varying degrees in six animals of this study (see also Fig. 1a); d) a gradual decrease after the marker peak (*Testudo graeca* 2) as seen in 2 animals of this study.

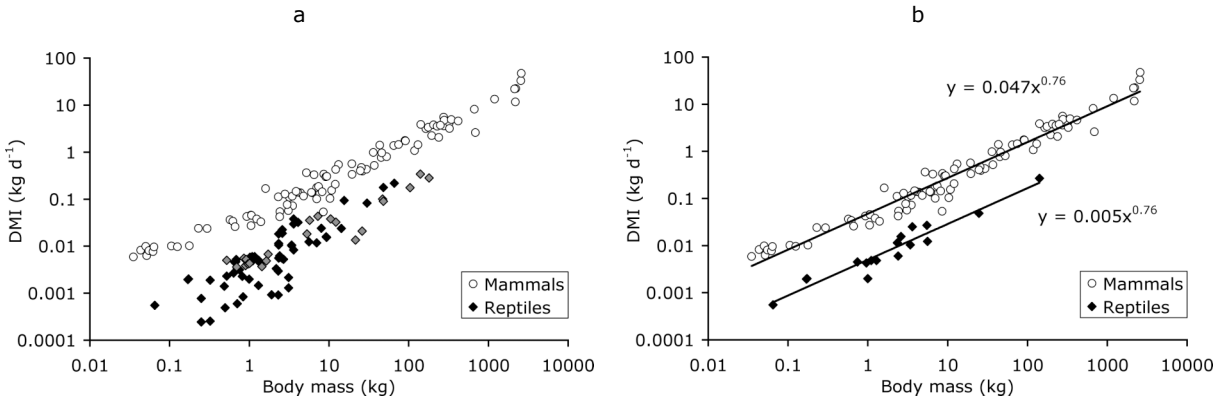

Fig. 2. Relationship between body mass (BM, kg) and dry matter intake (DMI, kg d^{-1}) in herbivorous reptiles and mammals. a) all available data for reptiles from the literature and this study on an individual basis; own measurements in grey. b) calculated species means. Regression equations for reptiles in a) is 0.003 (*95%CI* 0.003-0.004) $BM^{0.87\ (95\%CI\ 0.76\text{-}0.97)}$ (n=85, r^2=0.76, p<0.001) and in b) 0.005 (*95%CI* 0.004-0.006) $BM^{0.76\ (95\%CI\ 0.64\text{-}0.88)}$ (n=17, r^2=0.92, p<0.001). Species means for mammals from Clauss et al. (2007a) with the regression equation 0.047 (*95%CI* 0.042-0.053) $BM^{0.76\ (95\%CI\ 0.73\text{-}0.79)}$ (n=93, r^2=0.96, p<0.001). Reptile data from this study and literature sources (see methods).

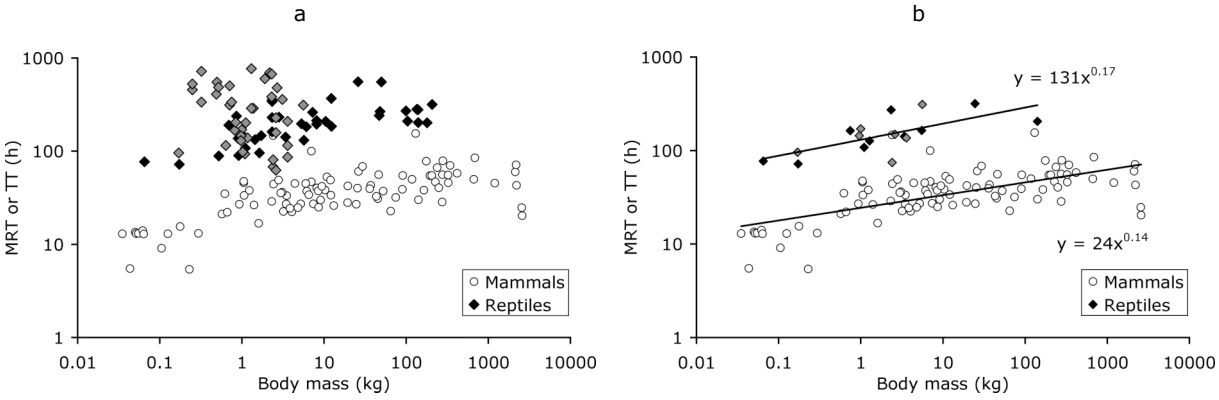

Fig. 3. Relationship between body mass (BM, kg) and mean retention time (MRT, black symbols) and transit time (TT, grey symbols) in herbivorous reptiles and mammals. a) all available individual data for reptiles; b) species means for reptiles (when both MRT and TT were given for a species, only MRT data were used) with the regression equation 131 (*95%CI* 108-158) $BM^{0.17\ (95\%CI\ 0.07\text{-}0.27)}$ (n=93, r^2=0.42, p<0.001). Species means for mammals from Clauss et al. (2007a) with the regression equation 24 (*95%CI* 22-28) $BM^{0.14\ (95\%CI\ 0.10\text{-}0.17)}$ (n=93, r^2=0.42, p<0.001). Reptile data from this study (MRT) and literature sources for MRT and TT (see methods).

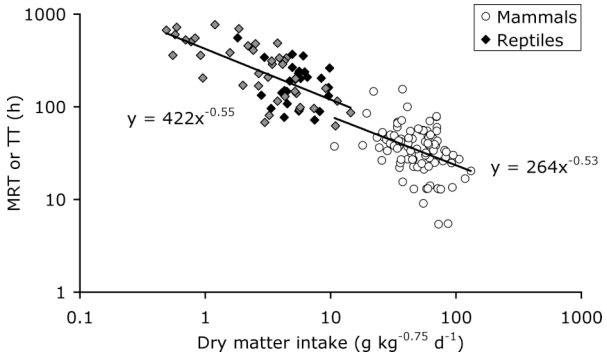

Fig. 4. Relationship between relative dry matter intake (rDMI, g $kg^{-0.75}$ d^{-1}) and particle mean retention time (MRT, black symbols) or transit time (TT, grey symbols) in individual herbivorous reptiles compared to species means for mammals. The regression equation for reptiles is 422 (*95%CI* 338-527) $BM^{-0.55}$ (*95%CI* -0.70- -0.39) (*n*=70, r^2=0.44, *p*<0.001). Mammal data from Clauss et al. (2007a) with the regression equation 264 (*95%CI* 94-739) $BM^{-0.53}$ (*95%CI* -0.79- -0.26) (*n*=93, r^2=0.15, *p*<0.001). Reptile data from this study (MRT) and literature sources for MRT and TT (see methods).

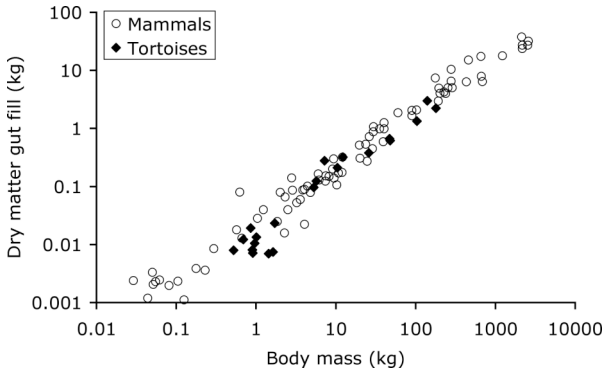

Fig. 5. Relationship between body mass (BM, kg) and dry matter gut fill (kg) calculated from intake, digesta retention and digestibility data (Holleman and White 1989). Data for tortoises from this study with a regression equation of 0.013 (*95%CI* 0.009-0.017) $BM^{1.07}$ (*95%CI* 0.95-1.19) (*n*=21, r^2=0.95, *p*<0.001). Mammal data are species averages from Müller et al. (in prep.) with the regression equation 0.024 (*95%CI* 0.022-0.029) $BM^{0.94}$ (*95%CI* 0.90-0.97) (*n*=80, r^2=0.97, *p*<0.001).

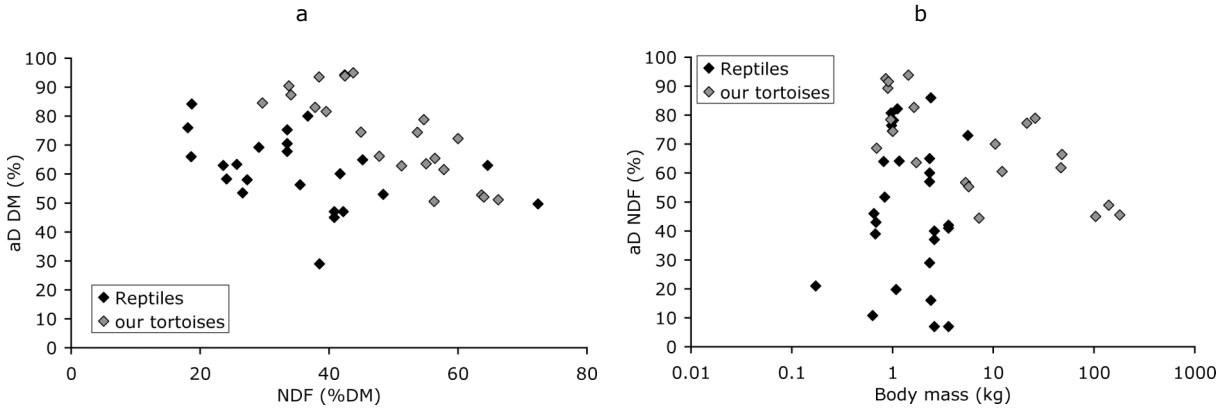

Fig. 6. Relationship between a) dietary neutral detergent fibre (NDF, %dry matter) and the apparent digestibility (aD, %) of dry matter (DM) and b) body mass (BM) and the aD NDF in herbivorous reptiles. Data from this study in grey.

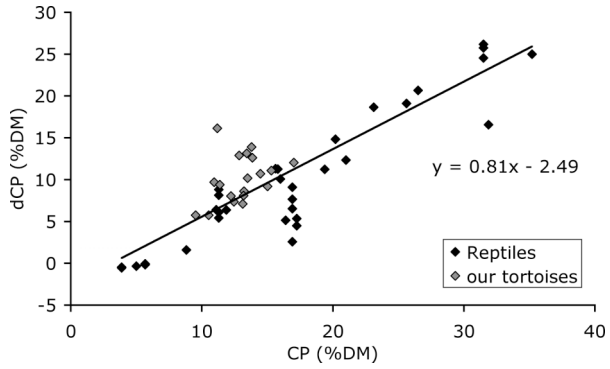

Fig. 7. Relationship between dietary crude protein content (CP, %dry matter) and digestible crude protein content (dCP, %DM) in herbivorous reptiles (tortoises from this study in grey) from this study and the literature. The linear regression equation is 0.81 (*95%CI* 0.69-0.93) CP – 2.49 (*95%CI* -4.51- -0.48). Data from this study and literature (see methods).

Chapter 3

Methane Production and body size in ruminants and equids

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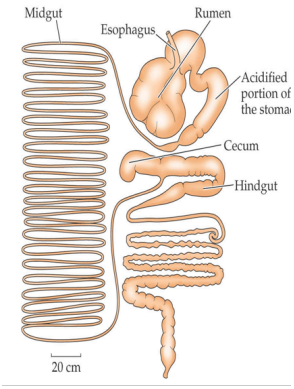
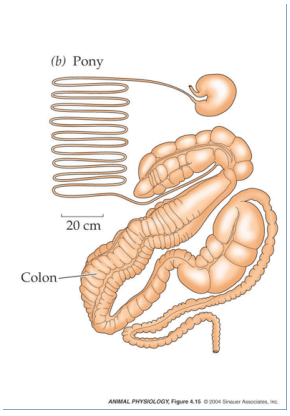
Evolutionary Ecology Research





Methane production in relation to body mass of ruminants and equids

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ABSTRACT

Background: It is generally assumed that ruminants produce more methane during digestion than equids, but direct comparisons of methane production when feeding on the same diet, or hat consider effects of allometric scaling, are missing.

Methods: We conducted an experiment with three sheep (*Ovis orientalis aries,* 94 ± 4 kg) and three Shetland ponies (*Equus ferus caballus*, 97 ± 6 kg) with ad libitum access to the same batch of grass hay, and measured feed intake, methane production (by using respiratory chambers), and calculated gut fill and feed digestibility. Data were added to a collection of data on methane production (L d^{-1}) of roughage-only fed ruminants (body mass range 26-610 kg) and equids (90-850 kg).

Results: Daily dry matter (DM) intake and DM digestibility were 39 ± 10 g $kg^{-0.75}$ d^{-1} and 48 ± 2 % in sheep and 72 ± 16 g $kg^{-0.75}$ d^{-1} and 41 ± 3 % in ponies; the calculated DM gut fill was 2.0 ± 0.5 % of body mass in sheep and 1.9 ± 0.4 % in ponies. Methane production was higher in sheep (30.3 ± 3.0 L d^{-1}) than in ponies (13.4 ± 4.6 L d^{-1}), representing 6.7 ± 1.7 and 1.5 ± 0.2 % of gross energy intake, respectively. The data collection showed a linear increase of methane production with body mass (i.e., BM^{1}) in both groups (with ruminants at a level 3.6 times higher than with equids).

Conclusions: Combined with the well-accepted assumption of energy and food intake scaling with metabolic body mass (i.e., $BM^{0.75}$), this leads to the conclusion that energetic losses due to methane (as a proportion of overall energy intake) increase with increasing body mass. The magnitude of the losses could limit the possible range of body sizes of ruminants but not of equids. Additionally, this finding adds to the growing body of evidence that relativizes a digestive advantage of large body size.

Keywords: herbivory, ruminant, hindgut fermenter, energetic losses, digestive physiology, excretion pattern, body size.

INTRODUCTION

Methane production is one of the unavoidable side-effects of vertebrate herbivory (Hackstein & Van Alen, 1996). Methanogenic microorganisms – members of the domain of the *Archaea* – are part of the microbial ecosystems present in the fermentation chambers of the gastrointestinal tracts of herbivores (Stevens & Hume, 1998). *Archaea* act as hydrogen sinks, converting H_2 and CO_2 to methane, thus keeping the partial pressure of H_2 low; this enhances the activity of fermenting microorganisms in the gut ecosystems (Jensen, 1996). It is generally accepted that methane production in ruminants is higher than in other herbivores such as hindgut fermenters (e.g. equids) or non-ruminant foregut fermenters (e.g. kangaroos) (Crutzen *et al.*, 1986; Clauss *et al.*, 2010). This might be attributed either to higher counts of *Archaea* in the rumen as the major fermentation chamber of ruminants (Morvan *et al.*, 1996) or to a higher prevalence of other hydrogen sinks such as reductive acetogenesis in hindgut fermenters (Prins & Lankhorst, 1977; Fievez *et al.*, 2001).

There has been a shift of the focus in research on methane production in herbivores, from concerns about methane representing a significant feed energy loss in the animal to methane as greenhouse gas thus contributing to global warming (Ellis *et al.*, 2007), resulting in a massive body of agricultural research. Comparative aspects of methane production have so far mostly been of interest for completing the estimation of national or global greenhouse gas inventories with respect to the contribution of non-ruminant domestic and free-ranging herbivores (e.g.

Crutzen *et al.*, 1986; Vermorel, 1997). In contrast, evolutionary or ecophysiological aspects of methane production have received little attention (Clauss & Hummel, 2005). This is best exemplified by the fact that the relationship between body mass (BM) and methane production has hardly been investigated. Such a relationship has so far only been reported as secondary findings in sheep (Pelchen & Peters, 1998) and cattle (Pavao-Zuckerman *et al.*, 1999).

Approaches to determine factors of influence on methane production in domestic ruminants have focused on the dietary composition of feed and plant secondary metabolites (Beauchemin *et al.*, 2008), feeding/intake levels (Ellis *et al.*, 2007), pasture management (e.g. DeRamus *et al.*, 2003), genotype and selection (Estermann *et al.*, 2002; Münger & Kreuzer, 2008) – factors that can be influenced by agricultural management practices.

Body size limitations due to methane production have been proposed for large herbivores (Prins & Kreulen, 1991; Van Soest, 1994). These considerations do not refer to the methane production usually observed in herbivores, which is due to a group of fast-growing *Archaea* that use H_2 and CO_2. They address another group of slow-growing *Archaea* that use acetate – one of the major fermentation products of gut *Bacteria*, and an important energy source for the vertebrate host – and convert it to methane, thus theoretically depriving the herbivore of one of its most important energy resources. These slow-growing *Archaea* have a generation time of approximately 4 days (Van Soest, 1994). If ingesta retention time is assumed to increase systematically with BM, there should be a body size threshold above which retention times in the fermentation chamber exceed this 4-day limit. Then, energetic losses due to acetate-based methanogenesis would theoretically become prohibitive. Prins and Kreulen (1991) presented a model calculating a maximum possible BM for ruminants of 1 to 1.5 metric tons. However, the validity of this concept is doubtful, given the facts that ingesta retention does not increase systematically with BM in large

herbivores, and that ingesta retention times exceeding 4 days have been measured in several vertebrate herbivores such as koalas (*Phascolarctos cinereus*), dugongs (*Dugong dugon*), sloths (*Bradypus tridactylus*) (reviewed in Clauss *et al.*, 2007) and land tortoises (Hatt *et al.*, 2002). In contrast, the question whether methane production due to faster-growing *Archaea* could impose a body size limit or a digestive disadvantage at increasing body size has so far not been addressed.

We compared methane production in ruminants and horses of similar size on the same diet, and added the results to literature data measured in ruminants and equids fed on roughage-only diets, to test for a scaling of methane production with BM. In particular, we expected that the resulting scaling relationship would either be similar to that of the volume of gut contents (scaling linearly with BM, i.e. $BM^{1.0}$), or similar to energy / food intake (scaling with $BM^{0.75}$), or similar to ingesta retention time (expected to result in no evident scaling with BM) (Clauss *et al.*, 2007).

METHODS

Three adult female sheep (*Ovis orientalis aries,* 94 ± 4 kg) and three adult female mini Shetland ponies (*Equus ferus caballus*, 97 ± 6 kg) were housed individually and were offered at *ad libitum* access to grass hay originating from one batch exclusively. This hay contained (g/kg dry matter (DM)): organic matter, 803; crude protein, 58; neutral detergent fibre (NDF), 582; acid detergent fibre, 326; acid detergent lignin, 46; gross energy 15.9 (MJ/kg DM). After an adaptation period of 2 weeks, the hay offered and the refusals were weighed daily, and faeces were completely collected at regular intervals (from 4 h at the beginning up to 12 h on the last day) for 7 days. Representative subsamples of the hay were analysed for contents of DM, nutrients and gross energy using standard laboratory methods (AOAC, 1997). In the faeces DM, NDF and

combustion energy were determined, and digestibilities of DM, NDF and energy were calculated. Mean ingesta retention times (MRT) were determined as part of a larger comparative study (Steuer et al., submitted) by feeding a particle (chromium-mordanted fibre, <2mm) marker prepared according to Udén et al. (1980); analyses and calculations of MRT were performed as described by Behrend et al. (2004). Gut DM fill was estimated using the exponential model of Holleman and White (1989). Following the 7-days collection period, animals were placed for two consecutive 22.5 h-periods into open circuit respiration chambers constructed and operated as described in Soliva and Hess (2007). The two chambers had a volume of 4.55 m^3 and provided constant humidity (60%), temperature (20 ±1°C), air flow (7.3 ± 0.1 m^3 h^{-1}), and pressure (987 ± 8 hPa). Gas analysers were manually calibrated with calibration gases (calibration gas 1: pure nitrogen (N_2), calibration gas 2: 20.44% mol oxygen, 0.439% mol carbon dioxide, 75.7 ppm mol CH_4). A possible drift of the analyser was numerically adjusted by performing repeated measurements of the outside air and calibration gases besides measurements of the chamber air composition. Methane concentrations were measured on a Binos 1001 (Fisher-Rosemount, Baar-Walterswil, Switzerland). Gas volumes were corrected for standard conditions (1013 hPa, 0 °C, 0% relative humidity). Methane production was expressed in absolute values and in relation to food intake, energy intake, and the intake of digestible energy and digestible NDF (as a measure of fibre).

The results from the present experiment were added to a literature collection of data on methane production in ruminants and equids of known BM fed roughage-only diets (n=57 with a BM range of 26-610 kg for ruminants, and n=20 with a BM range of 208-850 kg for horses; sources see Fig. 1) and a dataset on pigs from one study where the same diet was used over a BM range of 23-113 kg (Christensen & Thorbek, 1987). Experimental data for the sheep and

ponies were compared by t-test. The data collection was statistically analysed, after transforming the BM and the methane data by the natural logarithm, using regression analysis and a General Linear Model (GLM) with methane production as the dependent variable, species group (ruminants, equids, pigs) as a factor and BM as covariate (the species × BM interaction was not significant) using PSAW 18.0 (SPSS Inc., Chicago, IL). The significance level was set to 0.05.

RESULTS

The hay intake of the sheep was little more than half of that of the horses (39 ± 10 vs. 72 ± 16 g $kg^{-0.75}$ d^{-1}; Table 1). Additionally, sheep had 1.8 times longer mean particle retention times (54 ± 4 vs. 26 ± 1 h), 1.2 times higher DM digestibilities (48 ± 2 vs. 41 ± 3 %), and a more than twofold higher methane production (30.3 ± 3.0 vs. 13.4 ± 4.6 L d^{-1}). Yet, the calculated total gut fill was similar in sheep (1.9 ± 0.5 kg DM or 2.0 ± 0.5 % of BM) and horses (1.9 ± 0.5 kg DM or 1.9 ± 0.4 % of BM). In sheep, methane output represented 6.7 ± 1.7 and 12.3 ± 3.1 % of gross energy and digestible energy intake, respectively, whereas it represented 1.5 ± 0.2 and 3.2 ± 0.7 % in horses. The sheep produced three times more methane per unit of digested fibre (digestible NDF) than the horses (92 ± 15 vs. 28 ± 9 L kg^{-1}).

Available data on methane production in ruminants and equids suggest a systematic increase in methane output with BM (Fig. 1). The few data available for South American camelids suggest a similar methane production as in ruminants. Growing pigs also showed an increase of methane output with body mass in the study included (Fig. 1). The scaling of methane production (in L d^{-1}) was

$0.66 \times BM^{0.97}$ (r^2=0.87; p<0.001; n=61; *95% confidence interval (CI)* for exponent 0.88-1.07) in ruminants,

$0.18 \times BM^{0.97}$ (r^2=0.76; p<0.001; n=23; *95% CI* for exponent 0.72-1.22) in horses,

$0.07 \times BM^{0.99}$ (r^2=0.93; p<0.001; n=12; *95% CI* for exponent 0.79-1.19) in pigs.

In the GLM where methane production was considered as dependent variable, both BM (F=205.2, p<0.001) and species group (F=6.06, p=0.003) were significant.

When expressed per unit of food intake (Fig. 2a) or as a proportion of gross energy intake (Fig. 2b), the data indicate a slight but significant increase with BM in ruminants:

Methane production (in L kg^{-1} dry matter intake) was

$16.6 \times BM^{0.12}$ (r^2=0.25; p<0.001; n=45; *95% CI* for exponent 0.06-0.18).

Methane production (in % gross energy intake) was

$3.5 \times BM^{0.13}$ (r^2=0.25; p<0.001; n=44; *95% CI* for exponent 0.06-0.20).

For horses, the resulting exponents for the scaling of methane production per unit of DM and gross energy intake were 0.26 and 0.17, respectively; the *95% CI* of these exponents, however, included 0 in both cases, i.e. the regressions were not significant:

Methane production (in L kg^{-1} dry matter intake)

$2.0 \times BM^{0.26}$ (r^2=0.29; p=0.056; n=13; *95% CI* for exponent -0.01-0.53).

Methane production (in % gross energy intake)

$0.7 \times BM^{0.17}$ (r^2=0.16; p=0.171; n=13; *95% CI* for exponent -0.09-0.42).

DISCUSSION

Our results are consistent with the knowledge that methane losses constitute about 6-10% of the gross energy intake in forage-fed ruminants (Immig, 1996), with the average being very close to the default value of 6.5% assumed by the IPCC (2006), and that horses produce less methane than ruminants (Crutzen *et al.*, 1986). Ideally, all comparisons should be made on the basis of

digested plant cell wall or at least digestible energy, to rule out the possibility that differences in methane production are simply an effect of the amount of digested material taken up by the animal. The comparisons of methane production as a proportion of digestible energy or per unit of digested plant fibre in Table 1 indicate that even when comparing data on such a basis, systematic differences between ruminants and horses remain. These are strong indications for systematic differences in the microbial ecosystem between the species.

The collection of literature data demonstrates that a 100 kg-ruminant may have a similar methane output as a 400 kg-horse. The horses were comparatively poorer utilizers of the nutrients in the roughage and therefore had to ingest higher amounts than the sheep. This was associated with a shorter ingesta retention time in the horses but at a similar calculated DM gut fill in the two species. This means that ruminants have a significantly higher methane production compared to equids even under the condition of a similar gut fill, and could be due to several factors (Vermorel *et al.*, 1997a). Ingesta retention in ruminants is longer than in horses (Foose, 1982; Pearson *et al.*, 2006; cf. Table 1) and hence gives the *Archaea* more time to produce methane. Accordingly, methane production was shown to be related to ingesta retention time in ruminants (Okine *et al.*, 1989; Pinares-Patiño *et al.*, 2003a). Thus, assuming that retention time is an important factor, methane production should be high as well in hindgut fermenters, such as rhinoceroses, and non-ruminant foregut fermenters, such as hippopotamus, which have ingesta retention times of the same magnitude as ruminants (Clauss *et al.*, 2004; Clauss *et al.*, 2005; Steuer *et al.*, 2010). This assumption remains to be investigated.

The microbial profile in the fermentation chambers of the digestive tract differs between ruminants and horses. Horses have lower concentrations of *Protozoa* (Kern *et al.*, 1974) and *Archaea* (Morvan *et al.*, 1996) in the hindgut than ruminants have in their main fermentation

chamber, the rumen. The putative effect of these differences is evident in the higher methane production of ruminants; the actual causes for the differences in the microbial gut ecosystem – the reason why rumination is apparently linked to such a high methane output - remain to be elucidated. Again, if it is hypothesized that the well-known differences in retention time and in the amount of non-microbial digestion of non-fibre carbohydrates suffice to explain the observed differences in methane production between ruminants and equids, then similar high levels of methane production as in ruminants should be observed in other non-ruminant foregut fermenters such as hippopotamuses, peccaries, sloths, macropods or even colobine monkeys. At least in the case of the macropods, the scarce existing evidence suggests that this is not the case (Kempton *et al.*, 1976; von Engelhardt *et al.*, 1978; Dellow *et al.*, 1988).

With an even lower contribution of microbial fermentation to the overall energy gain from feed compared to the horses, pigs potentially have an even lower methane output at the same BM and gut fill, but this remains to be investigated on roughage-only diets or diets resembling the natural diet of suids. Existing data in domestic pigs suggest that high-fibre diets lead to an increase in methane production compared to the commonly fed low-fibre diets (Kirchgessner *et al.*, 1991).

Whether methane production increases systematically with BM has so far only been suggested (Clauss & Hummel, 2005) or observed incidentally in individual species (Christensen & Thorbek, 1987; Pelchen & Peters, 1998; Pavao-Zuckerman *et al.*, 1999), but has not been investigated systematically so far. In itself, this finding is not surprising, because larger animals consume more food; more methane production is expected as the absolute amount of processed food increases. Rather, the pattern of the increase – the scaling with BM – is of particular interest. A linear scaling of methane production with BM, as suggested by the regression

equations from our data collection (in contrast to the scaling with $BM^{0.75}$ as assumed by IPCC 2006, p. 10.28), has important consequences for general herbivore physiology and evolution. If this linear scaling can be confirmed in further studies, it would suggest that methane production is, across BM ranges within a digestion type (e.g. ruminant or equid), mainly a factor of gut capacity, as found within sheep (Pinares-Patiño *et al.*, 2003a). Gut capacity (measured as wet contents) has been shown repeatedly to scale linearly with BM (reviewed in Clauss *et al.*, 2007). Because food intake scales with $BM^{0.75}$ (reviewed in Clauss *et al.*, 2007), a linear scaling of methane production with BM would translate into increasing energetic losses due to methane per unit of food intake with increasing BM. This is illustrated by the relationship of relative methane production – either per unit food intake or per unit of energy intake – in Fig. 2. For horses, these relationships were not significant, potentially due to the comparatively low sample size. The magnitude of proportionate methane production in ruminants per unit of energy intake is so large that a limit in body size increase in this group can be expected by the action of fast-growing, H_2 and CO_2-using *Archaea* alone (with methane losses approaching on average 9% of gross energy intake at a BM of one metric ton). A digestive system like that of the equids, in contrast, would not reach the same limitation even when scaled up to body sizes of the largest presumed hindgut fermenters, the mammalian Indricotheres (15 tons, Fortelius & Kappelman, 1993) or the dinosaur sauropods (up to 100 tons, Sander *et al.*, 2010), with methane losses estimated at about 3.3-4.4% of gross energy intake. Whatever the causes of the increased methane production in ruminants are, its scaling with BM may be responsible for the different body size ranges achieved by ruminant and non-ruminant herbivores (Clauss *et al.*, 2003) and thus represent an intriguing example of a physiological constraint on the evolutionary history of a particular animal group.

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Table 1. Feed intake, digestion and methane production in sheep and horses of similar body mass.

		Sheep			Ponies			*p**
		1	2	3	1	2	3	
Body mass (BM)	(kg)	93	91	99	101	101	90	0.488
DM intake	(g $kg^{-0.75}$ BM d^{-1})	45	28	43	76	85	54	0.039
Mean retention time	(h)	54	58	47	26	22	25	0.001
DM digestibility	(%)	47	51	46	41	39	45	0.041
Gut fill	(kg DM)	2.3	1.4	2.0	2.0	2.0	1.2	0.735
Methane	(L d^{-1})	33.7	28.1	29.1	16.9	15.1	8.3	0.006
	(L kg^{-1} DM intake)	24.8	34.4	21.6	7.0	5.6	5.3	0.006
	(% of GE)	6.2	8.5	5.4	1.7	1.4	1.3	0.005
	(% of DE)	10.0	15.8	11.0	4.0	2.9	2.8	0.008
	(L kg^{-1} dNDF)	76	107	93	39	24	23	0.003

DM, dry matter; GE, gross energy; DE, digestible energy; dNDF, digestible neutral detergent fibre
*independent sample t-test comparing sheep and ponies

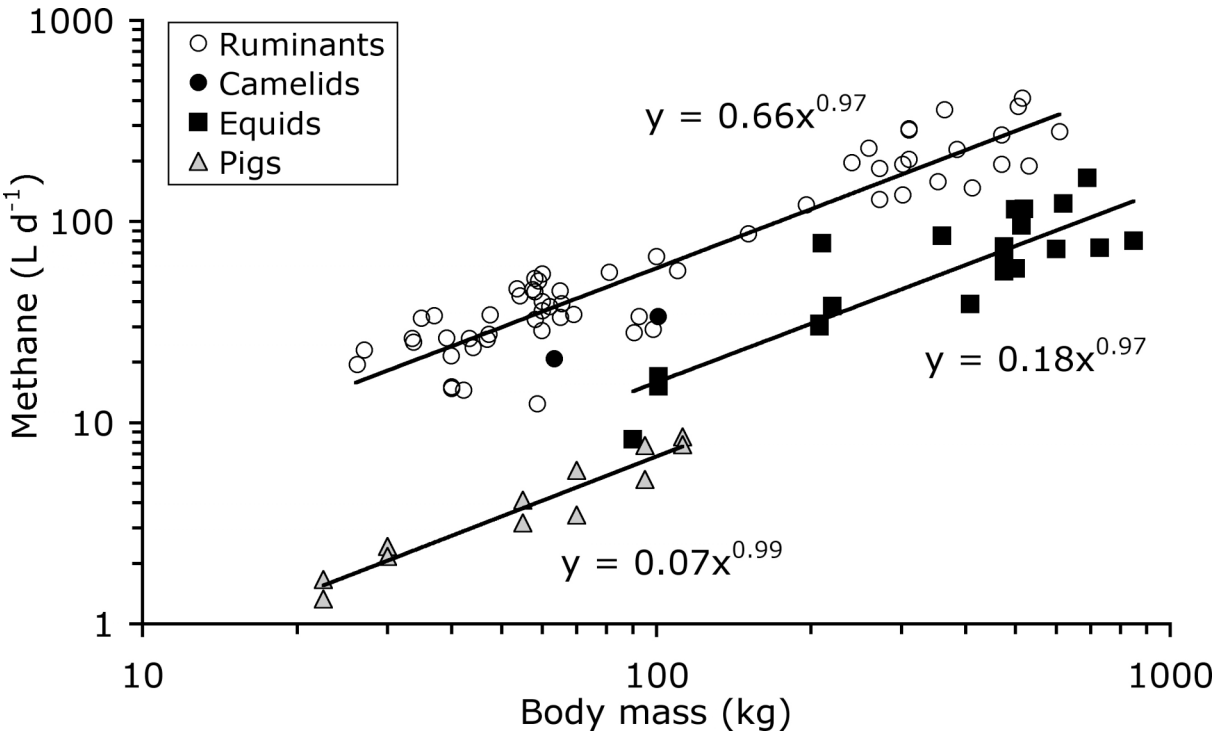

Fig. 1. Methane production in relation to body mass in ruminants (Ritzman & Benedict, 1938; Belyea *et al.*, 1985; Terada *et al.*, 1987; Margan *et al.*, 1988; Okine *et al.*, 1989; Hironaka *et al.*, 1996; Klita *et al.*, 1996; Vermorel, 1997; Vernet *et al.*, 1997; Galbraith *et al.*, 1998; Kurihara *et al.*, 1999; McCaughey *et al.*, 1999; Boadi & Wittenberg, 2002; Ulyatt *et al.*, 2002; Pinares-Patiño *et al.*, 2003a; Pinares-Patiño *et al.*, 2003b; Carulla *et al.*, 2005; Puchala *et al.*, 2005; Swainson *et al.*, 2007; Animut *et al.*, 2008; Tiemann *et al.*, 2008; Hart *et al.*, 2009; and this study), South American camelids (Vernet *et al.*, 1997; Pinares-Patiño *et al.*, 2003b), horses (Ritzman & Benedict, 1938; Nehring, 1956; Vermorel, 1997; Vermorel *et al.*, 1997a; Vermorel *et al.*, 1997b; and this study) and pigs (Christensen & Thorbek, 1987). See results section for regression equations.

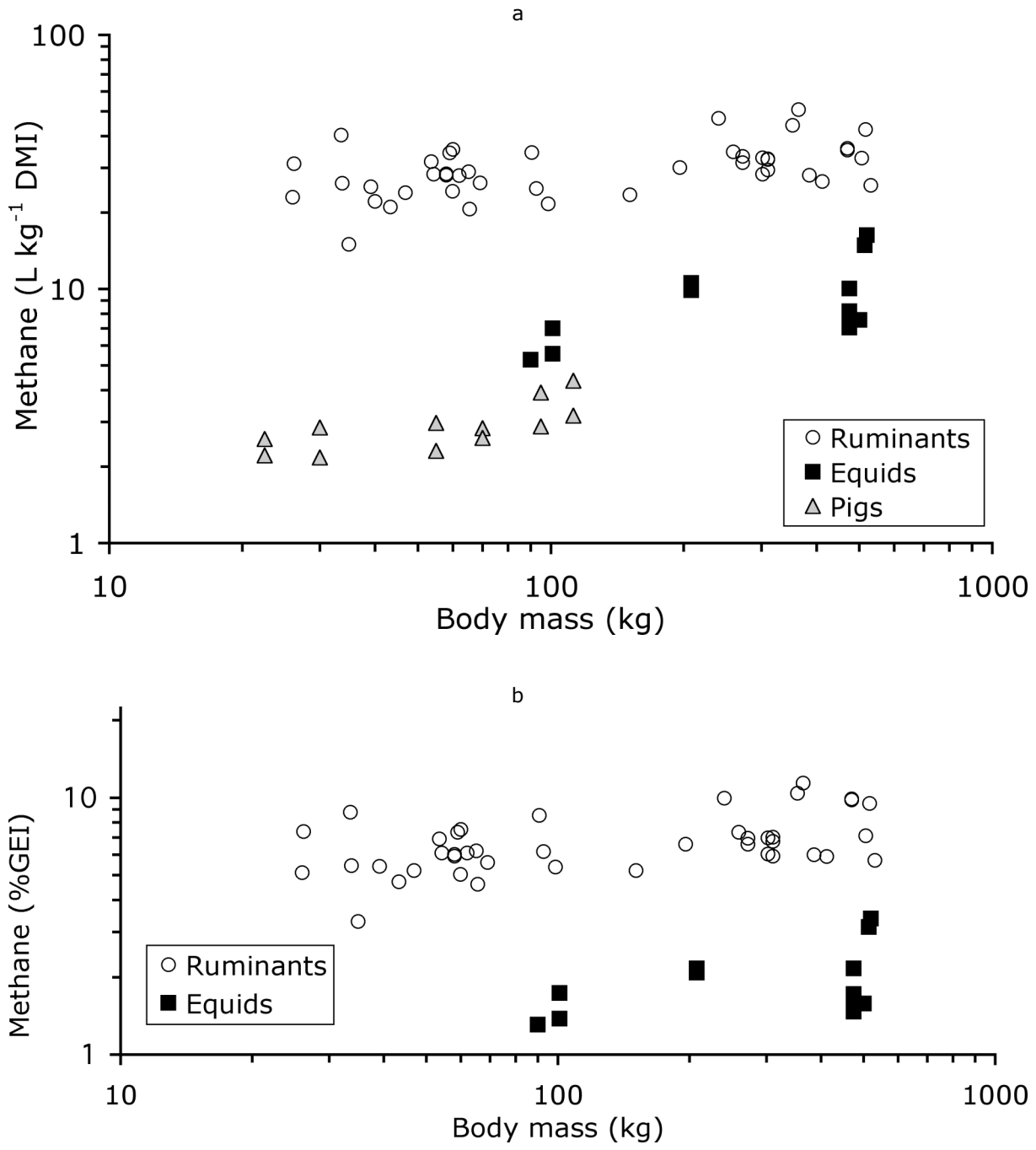

Fig. 2. Methane production a) per unit of dry matter intake (DMI) and b) per unit of gross energy intake (GEI) in relation to body mass in ruminants, horses and pigs. Same data sources as Fig. 1 (excluding those from which the respective measures could not be derived).

Chapter 4

Methane in rabbits (*Oryctolagus cuniculus*) and guinea pigs (*Cavia porcellus*) on a hay-only diet: implications for the scaling of methane procution with body mass in non-ruminant mammalian herbivores

accepted by

Comparative Biochemistry and Physiology A

Methane output of rabbits (*Oryctolagus cuniculus*) and guinea pigs (*Cavia porcellus*) fed a hay-only diet: implications for the scaling of methane production with body mass in non-ruminant mammalian herbivores

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A B S T R A C T

It is assumed that small herbivores produce negligible amounts of methane, but it is unclear whether this is a physiological peculiarity, or simply a scaling effect. A respiratory chamber experiment was conducted with six rabbits (*Oryctolagus cuniculus,* 1.57 ± 0.31 kg body mass) and six guinea pigs (*Cavia porcellus*, 0.79 ± 0.07 kg) offered grass hay *ad libitum*. Daily dry matter (DM) intake and DM digestibility were 50 ± 6 g $kg^{-0.75}$ d^{-1} and 55 ± 6 % in rabbits and 59 ± 11 g $kg^{-0.75}$ d^{-1} and 61 ± 3 % in guinea pigs, respectively. Methane production was similar for both species (0.20 ± 0.10 L d^{-1} and 0.22 ± 0.08 L d^{-1}) and represented 0.69 ± 0.32 and 1.03 ± 0.29 % of gross energy intake in rabbits and guinea pigs, respectively. In relation to body mass (BM) guinea pigs produced significantly more methane. The data on methane per unit of BM obtained in this study and from literature on methane output of elephant, wallabies and hyraxes all lay close to a regression line derived from roughage-fed horses, showing an increase in methane output with BM. The regression including all data was nearly identical to that based on the horse data only (methane production in horses [L d^{-1}] = 0.18 body mass [kg]$^{0.97\ (95\%CI\ 0.92–1.02)}$) and indicates linear scaling. Because feed intake typically scales to $BM^{0.75}$, linear scaling of methane output translates into increasing energetic losses at increasing BM. Accordingly, the data collection indicates that an increasing proportion of ingested gross energy is lost because relative methane production increases with BM. Different from ruminants, such losses (1-2% of gross energy) appear too small in non-ruminant herbivores to represent a physiologic constraint on body size. Nevertheless, this relationship may represent a physiological disadvantage with increasing herbivore body size.

1. Introduction

Methane production has been detected in the faeces of nearly all herbivorous and, additionally, some omnivorous and carnivorous terrestrial vertebrates (Hackstein and Van Alen 1996). Therefore, methanogenesis is considered a primitive-shared characteristic among reptiles, birds and mammals (Hackstein and Van Alen 1996; Mackie et al. 1999). *In vivo* methane production has been measured in large herbivores – predominantly domestic ruminants, but also domestic horses – in order to characterise feed efficiency and the contribution of agricultural systems to greenhouse gas production (reviewed in Franz et al. 2010b). In small herbivores and omnivores such as rabbits (*Oryctolagus cuniculus*), guinea pigs (*Cavia porcellus*), naked mole rats (*Heterocephalus glaber*), and rats (*Rattus rattus*), methanogenesis has been mainly studied by *in vitro* assays (Prins and Lankhorst 1977; Yahav and Buffenstein 1991; Piattoni et al. 1996; Marounek et al. 1997; Piattoni et al. 1997; Marounek et al. 1998; Piattoni et al. 1998; Marounek et al. 1999; Tsukahara and Ushida 2000). *In vivo* measurements are less common (Rodkey et al. 1972 - rabbits, guinea pigs and rats; McKay and Eastwood 1983 - rats; Dufour-Lescoat et al. 1995 - rats; Belenguer et al. 2008 - rabbits). In particular, measurements of methane production from small herbivores on roughage diets (mimicking the natural diet) are lacking so far. Such measurements are required to test whether methane production scales with body mass in a certain manner. Franz et al. (2010b) suggested that methane production scales linearly with body mass in horses and ruminants, and at a higher magnitude in the latter.

In order to test whether or not methane production per unit of body mass in small herbivores is of a similar magnitude as that of larger non-ruminant herbivores, methane was measured in rabbits and guinea pigs kept on a hay-only diet; subsequently, the results were added to a literature data collection.

2. Materials and methods

Six pygmy rabbits and six guinea pigs were housed individually at 20 ± 2°C on a 12 h light : 12 h dark schedule. The animals were offered *ad libitum* access to grass hay (organic matter, 926 g kg^{-1}; crude protein, 72 g kg^{-1}; neutral detergent fibre (NDF), 635 g kg^{-1}; acid detergent fibre, 360 g kg^{-1}; gross energy, 16.47 MJ kg^{-1}, on a dry matter (DM) basis, analysed in two subsamples by standard procedures (AOAC 1997). After an adaptation period of 2 weeks, feed intake (offered and leftover) was registered daily, and faeces were collected completely for 7 days. Faeces were dried and analysed for DM, NDF and gross energy (AOAC 1997). Due to a logistical error, faecal samples of only three rabbits were analysed for gross energy content. Subsequently, DM, NDF and energy digestibilities were calculated. Coprophagy was not prevented, or accounted for, in the present study. Fresh water was available at all times.

After the 7-day collection period, animals were placed in open circuit respiration chambers operated as described in Soliva and Hess (2007) for two consecutive 22.5 h periods. The chambers had a volume of 0.85 m^3 and provided constant humidity (60%), temperature (20 ± 1°C), airflow (1.00 ± 0.04 m^3 h^{-1}), and pressure (987 ± 8 hPa). Gas analysers were manually calibrated with calibration gases (calibration gas 1: pure nitrogen (N_2), calibration gas 2: 20.44% mol oxygen, 0.439% mol carbon dioxide, 75.7 ppm mol CH_4). A possible drift of the analyser was numerically adjusted by regularly analysing outside air and calibration gases besides measurements of the chamber air composition. Methane concentrations were measured on a Binos 1001 (Fisher-Rosemount, Baar-Walterswil, Switzerland). Gas volumes were corrected for standard conditions (1013 hPa, 0 °C, 0% relative humidity). Methane production was expressed in absolute values and in relation to intakes of food, gross energy, digestible energy and

digestible NDF (as a measure of fibre). Comparisons between rabbits and guinea pigs were performed using a t-test.

The results from the present experiment were added to a literature collection of data on methane production in ruminants and equids of known body mass fed roughage-only diets (Franz et al. 2010b), and data on an elephant (*Elephas maximus*) (Benedict 1936), on tammar wallabies (*Macropus eugenii*) and on hyraxes (*Procavia habessinica*) (von Engelhardt et al. 1978). This data collection was analysed after ln-transformation using regression analysis with PSAW 18.0 (SPSS Inc., Chicago, IL). The significance level was set to 0.05.

3. Results and discussion

3.1. Methane output of non-ruminant mammalian herbivores

The rabbits had higher body masses but a lower methane production per unit of body mass (Table 1). No other differences between the species were significant, although relative feed intake (per unit metabolic body mass) as well as the relative measures of methane output (per unit of feed or gross energy intake) all tended towards significance. The only exception was methane output expressed per unit digestible fibre intake, which was very similar in both species. In the direct comparison, guinea pigs therefore had a higher methane output per unit body mass than rabbits. The amount of methane produced by the guinea pigs in the present study was very similar to that reported by Rodkey et al. (1972) in animals whose diet was not specified (Fig. 1). In contrast, the amount of methane produced in the rabbits in the present study was distinctively higher than the levels reported by Belenguer et al. (2008). This discrepancy is striking; the animals used in the present study had been exposed to grass hay as a dietary item throughout their lives, which might not have been the case with the animals used in the other study. In

rabbits, *in vitro* evidence suggests that methane production is age-dependent (Piattoni et al. 1996; Marounek et al. 1999). The animals used by Belenguer et al. (2008) for *in vivo* measurements were much younger than those used in the present study (2.5 months *vs*. >1 year). Thus, differences in age and diet most likely explain the observed differences.

Guinea pigs usually achieve higher digestibility coefficients than rabbits (Slade and Hintz 1969; Sakaguchi et al. 1987). This is consistent with the non-significant trend in DM digestibility noted in the present study. The comparative literature on the digestive physiology of rabbits and guinea pigs (reviewed in Franz et al. 2010a) suggests a higher contribution of microbial fermentation to the overall digestion in guinea pigs, which would also explain the observed higher methane production in the guinea pigs as compared to the rabbits when expressed per unit of body mass. In contrast, rats, being omnivores not relying on microbial fermentation to the same extent as guinea pigs and rabbits, have a comparatively low relative methane output (Fig. 1). The rats in the study of Rodkey et al. (1972) only produced 24 ± 11 % of the expected values when compared to estimated values extrapolated from the general regression equation for non-ruminant herbivores ($0.18\ BM^{0.97}$; see below).

Empirical data for small herbivores indicates that reductive acetogenesis occurs to a significant extent in the hindgut (Prins and Lankhorst 1977). This could explain the relatively low level of methanogenesis in non-ruminant herbivores when compared to ruminants. Reductive acetogenesis has also been detected in a larger avian hindgut fermenter, the ostrich (Fievez et al. 2001). In the hindgut of domestic horses, lower concentrations of *Archaea* (methanogens) have been found than in the rumen of domestic ruminants (Morvan et al. 1996). Protozoa, which are hydrogen producers and therefore have a close metabolic relationship with *Archaea*, have been detected in the digestive tract of both guinea pigs (Dehority 1986) and

rabbits (Lelkes and Chang 1987). Similarly, protozoa have been described in the digestive tracts of elephants (Dehority 1986), equids (Kern et al. 1974), wallabies (Cameron 2003), and hyraxes (Schubats 1908).

4.2. Relationship of body mass and methane production in non-ruminant mammalian herbivores

Franz et al. (2010b) described the relationship between body mass and methane production (L d^{-1}) in horses as

0.18 $BM^{0.97}$ (r^2=0.76; p<0.001; n=23; *95%CI* factor 0.04-0.79; *95%CI* exponent 0.72-1.22).

The values for rabbits and guinea pigs from the present study, and for the other non-ruminant herbivores from the literature, were all close to this regression equation (Fig. 2). When combining the individual measurements for the non-ruminant herbivores, the resulting regression equation was

0.18 $BM^{0.97}$ (r^2=0.98; p<0.001; n=41; *95%CI* factor 0.14-0.23; *95%CI* exponent 0.92-1.02).

The inclusion of the additional data did not change the scaling relationship but reduced the magnitude of the confidence intervals.

In the data collection of Franz et al. (2010b), methane production of ruminants as a percentage of gross energy intake scaled with body mass as

3.53 $BM^{0.13}$ (r^2=0.25; p<0.001; n=44; *95%CI* factor 2.52-4.94; *95%CI* exponent 0.06-0.20).

For all non-ruminant herbivores combined (Fig. 3a), this relationship was

0.79 $BM^{0.15}$ (r^2=0.57; p<0.001; n=25; *95%CI* factor 0.63-0.99; *95%CI* exponent 0.09-0.20). Thus, the exponent of the scaling relationship was not significantly different between ruminants and non-ruminants (overlapping *95% CI*), but the scaling factor was.

When expressing methane losses as a percentage of digestible energy intake (Fig. 3b), the scaling in ruminants was nearly significant at

7.87 $BM^{0.09}$ (r^2=0.11; p=0.053; n=35; *95%CI* factor 5.13-12.06; *95%CI* exponent -0.001-0.18).

For all non-ruminant herbivores combined, this relationship was significant with

1.48 $BM^{0.17}$ (r^2=0.71; p<0.001; n=31; *95%CI* factor 1.21-1.81; *95%CI* exponent 0.13-0.21). When the rabbits were excluded because of their different digestive strategy for hindgut fermenters (Franz et al. 2010a), then the resulting relationship was significant at

1.83 $BM^{0.13}$ (r^2=0.70; p<0.001; n=28; *95%CI* factor 1.53-2.20; *95%CI* exponent 0.10-0.17).

Overall, these results suggest that methane output, in a broad-scale comparison, scales linearly with body mass in non-ruminant mammalian herbivores across a large range of body sizes (Fig. 2). This translates into an increase of energy losses due to methane as a proportion of overall energy intake with body mass (Fig. 3). This is obvious even though at a fine resolution, differences in digestive physiology like those found between guinea pigs and rabbits also influence methane production. The few existing data on a non-ruminant foregut fermenter, the wallaby (a macropod), suggest that it is rumination, and not foregut fermentation as such, that makes the difference with respect to methane production (Clauss et al. 2010). Although *in vivo* measurements on methane production in macropods are scarce (Kempton et al. 1976; von Engelhardt et al. 1978; Dellow et al. 1988), and data from only one study could actually be included in this comparative evaluation, it has recently been claimed that macropods produce little methane (Wilson and Edwards 2008). We suggest that this is not due to a specific particularity of macropods, but just within the scope of methane production observed in other non-ruminant herbivores.

The scaling of methane production with body mass adds to the assumption of Clauss and Hummel (2005) that, contrary to previous concepts, an increase in body mass does not necessarily translate into a digestive advantage. Nevertheless, given the comparatively low level of methane production in non-ruminant herbivores, it is questionable whether methane output would ever reach a relevant proportion of overall energy intake. If the regression equations for non-ruminants are extrapolated to body masses of the largest terrestrial herbivores ever – the sauropod dinosaurs that reached up to 100 metric tonnes (Sander et al. 2010) –, the resulting proportion of methane of 4.4 % of gross energy intake can probably not be regarded as a physiological limit of body size (as compared to 6-10 % observed in ruminants). When considering methane as a proportion of digestible energy, however, extrapolated values (with any of the two equations either including or excluding rabbits) at 100 metric tonnes would correspond to 8.2-10.5 % of digestible energy intake in non-ruminant herbivores, and thus reach values observed in ruminants today. If one accepts the concept that methane production represents a physiological limitation to body size evolution in ruminants, then very large sauropods could be hypothesized to have reached a similar constraint.

4.3. Conclusions

The data collection of the present study suggests that energy losses through methane production increase – though only slightly – with increasing body mass in non-ruminant mammalian herbivores. On a larger scale, this is overriding the differences between individual species, such as the rabbits and guinea pigs of the present study. More detailed *in vivo* studies on a wide range of herbivore species are needed to identify differences between groups characterized by a specific taxonomy or digestive physiology.

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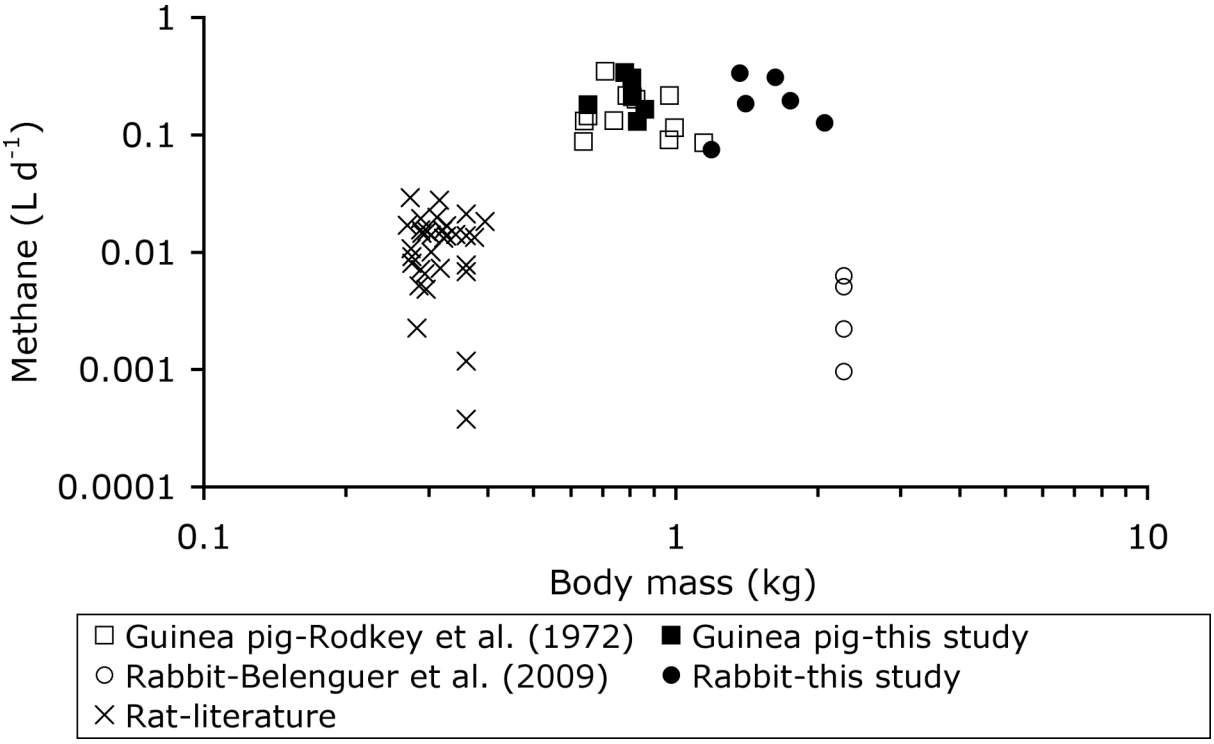
Legend of the figures:

Fig. 1. Relationship between body mass and methane production in guinea pigs using data from the present study and from Rodkey et al. (1972), in rabbits using data from the present study and from Belenguer et al. (2008), and in rats using datasets from Rodkey et al. (1972) and McKay and Eastwood (1983).

Fig. 2. Relationship between body mass and methane production (in litres per day) in ruminants (grey regression line from Franz et al. 2010b) and non-ruminant mammalian herbivores. Rabbit and guinea pig data are from the present study, horse data collected in Franz et al. (2010b), data on the elephant are from Benedict (1936). Data on wallabies (fed on roughage) and hyraxes (fed on a mixed diet, excluding one outlier with very low methane production) originate from von Engelhardt et al. (1978). The black regression line was exclusively calculated from the horse data, but was extrapolated to lower and higher body masses. All regression equations used are explained in text (results section).

Fig. 3. Relationship between body mass and methane production as a percentage of a) gross energy (GE) intake and b) digestible energy (DE) intake in ruminant and non-ruminant mammalian herbivores. Ruminant and horse data collected in Franz et al. (2010b), the rabbit and guinea pig data are from the present study. Regression equations depicted for ruminant (grey line) and non-ruminant herbivores with (gray line) and without rabbits (black line) are explained in text (results section).



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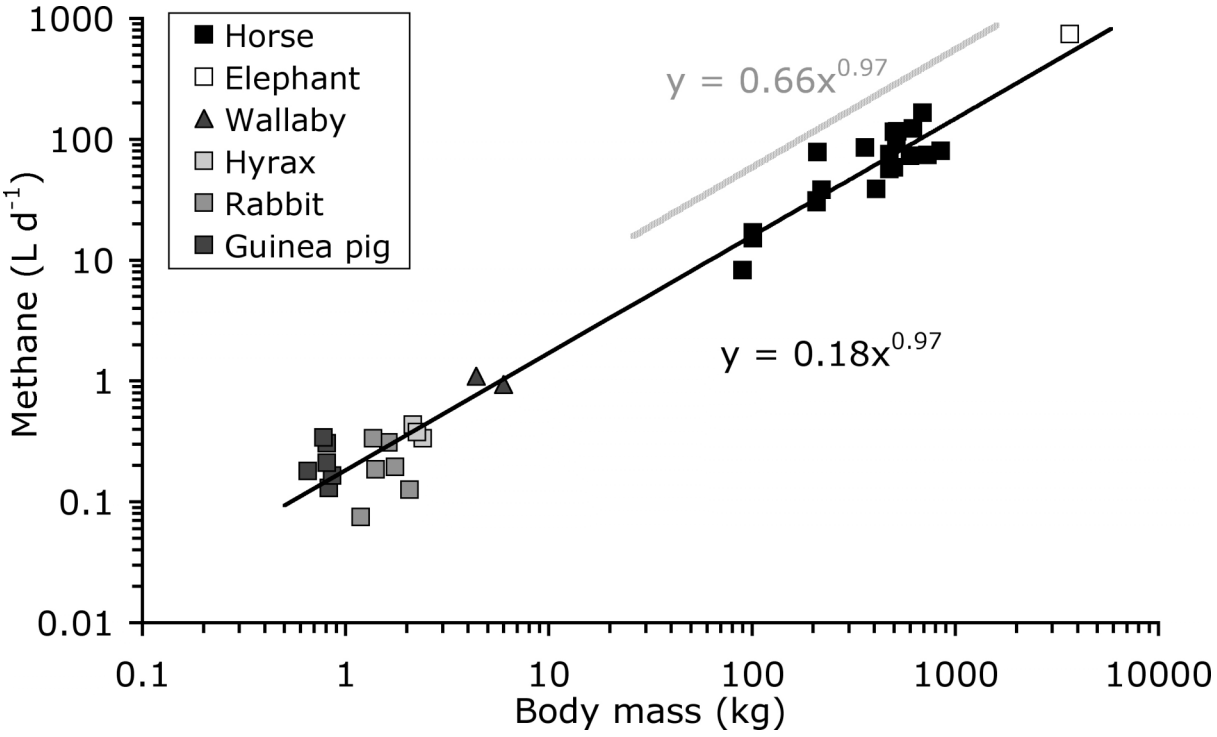

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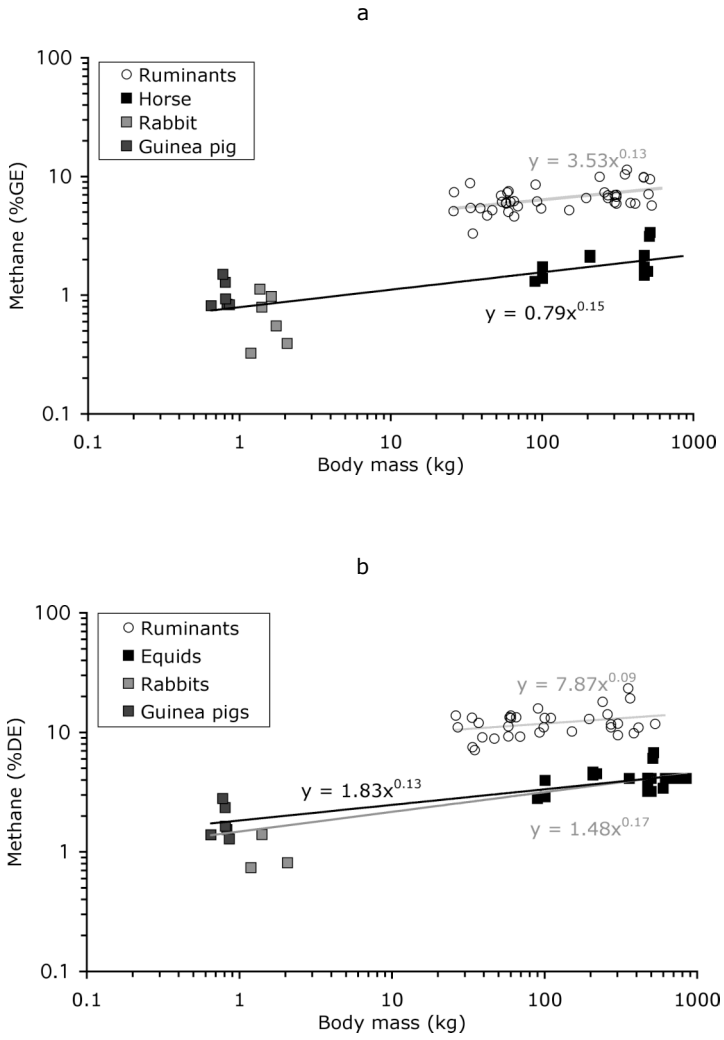

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Table 1
Mean (±SD) body mass, feed intake, digestibility and methane production in rabbits (*Oryctolagus cuniculus*, n=6) and guinea pigs (*Cavia porcellus*, n=6) on a hay-only diet.

		Rabbit	Guinea pig	P-value[a]
Body mass (BM)	kg	1.57 ± 0.31	0.79 ± 0.07	0.001
Dry matter intake (DMI)	g $kg^{-0.75}$ BM d^{-1}	50 ± 6	59 ± 11	0.076
Dry matter digestibility	%	55 ± 6	61 ± 3	0.075
Methane output	L d^{-1}	0.20 ± 0.10	0.22 ± 0.08	0.784
	L kg^{-1} BM d^{-1}	0.13 ± 0.07	0.28 ± 0.11	0.016
	L kg^{-1} DMI	2.93 ± 1.36	4.40 ± 1.23	0.076
	% of gross energy	0.69 ± 0.32	1.03 ± 0.29	0.084
	% of digestible energy	0.98 ± 0.36	1.83 ± 0.60	0.064
	L kg^{-1} digestible NDF[b]	10.7 ± 4.8	12.8 ± 4.1	0.450

[a] Independent sample t-test.
[a] NDF neutral detergent fibre.

Chapter 5

Methane output in herbivorous tortoises: energetic losses related to herbivore body mass

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Methane output in herbivorous tortoises: energetic losses related to herbivore body mass

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Abstract

An increase in body mass (BM) is traditionally considered an advantage for herbivores in terms of digestive efficiency. However, more recently, disadvantages of large BM such as increased methane losses have been discussed, and in mammals, a linear scaling of methane output with BM was described. In order to generate comparative data in a non-mammal herbivore group of a large BM range, we conducted feeding trails with 24 tortoises (*Testudo graeca*, *T. hermanni*, *Geochelone sulcata*, *G. nigra*, *Dipsochelys dussumieri*) ranging in body mass (BM) from 0.52 to 180 kg, fed a diet of grass hay ad libitum and salad. Mean daily dry matter and gross energy intake measured over 30 consecutive days scaled to $BM^{0.75\ (95\%CI\ 0.64\text{-}0.87)}$ and $BM^{0.77\ (95\%CI\ 0.66\text{-}0.88)}$, respectively. Methane production was measured over two consecutive days in respiration chambers; mean daily methane production scaled to $BM^{1.03\ (95\%CI:\ 0.84\text{-}1.22)}$. When expressed as energy loss in proportion of gross energy intake, methane losses accordingly scaled to 0.70 (*95%CI*: 0.47-1.05) $BM^{0.29\ (95\%CI:\ 0.14\text{-}0.45)}$. This scaling overlaps in its confidence intervals to that calculated for nonruminant mammals 0.79 (*95%CI*: 0.63-0.99) $BM^{0.15\ (95\%CI:\ 0.09\text{-}0.20)}$, but is lower than that for ruminants. The similarity between nonruminant mammals and tortoises suggest a convergent evolution of the gut microbial fauna in ectotherms and endotherms, and that the increase in energetic losses due to methane production with increasing body mass is a general allometric principle in herbivores. While not of a magnitude that must be considered constraining for BM in the observed range of extant tortoises or nonruminant herbivores, these findings add evidence to the suspicion that large body size itself does not necessarily convey a digestive advantage.

Introduction

Among the different advantages commonly linked to an increase in body size (Sander et al. 2010), a widespread concept is that of an increasing digestive efficiency in larger herbivores. Based on the observation that energetic requirements of animals scale to metabolic body weight (i.e., body mass$^{0.75}$) but gut capacity scales linearly with body mass (BM$^{1.0}$) in mammalian herbivores, Bell (1971) and Jarman (1974) deducted that at larger BM, more gut capacity was available per unit energy requirement/food intake. This so-called 'Jarman-Bell principle' (Geist 1974) was further refined subsequently (Parra 1978; Demment & Van Soest 1985; Illius & Gordon 1992) and has found widespread application in ecology (e.g. reviewed in Fleming 1991; Barboza & Bowyer 2000).

The attractiveness of this concept is that it provides an intuitive ultimate reason for the observation that larger-bodied herbivores usually ingest food of lower nutritional quality (Owen-Smith 1988; Codron et al. 2007). However, recent findings do not support the notion that digestibility (Pérez-Barberìa et al. 2004; Clauss et al. 2009) or ingesta retention (Clauss et al. 2007) increase systematically with body mass. Among potential disadvantages, ingesta particle size – one of the factors influencing digestive efficiency – increases with body mass (Fritz et al. 2009), and it has been suggested that energetic losses due to methane production is also higher in larger animals (Clauss & Hummel 2005).

Methane production has been mainly measured in domestic herbivores to address the issue of energetically efficient feeding or, more recently, the problem of greenhouse gas emission in agricultural systems; the research focuses on a reduction of methane production (Martin et al.

2010). The methane production of non-domestic species is mainly of interest to complete national or global methane budgets (Crutzen et al. 1986). In contrast, comparative investigations on methane production with respect to herbivore physiology are rare. Methane production has been demonstrated in faeces of captive specimens of nearly all herbivorous terrestrial herbivores, including reptiles (Hackstein & Van Alen 1996), and methanogenes have been demonstrated by fluorescence microscopy in land and marine iguanas (Mackie et al. 2004); but in vivo methane production has, so far, not been investigated in reptiles to our knowledge. Recently, Franz et al. (2010a; 2010b) presented data collections that suggest that methane production scales linearly with BM in ruminant and nonruminant mammalian herbivores. The implication of this finding is that because food intake scales to $BM^{0.75}$, energetic losses due to methane increase per unit ingested food with increasing body size. Thus, methane production might actually represent another factor that does not become more advantageous for larger herbivores.

In order to further test this concept, we chose specimens of another clade of herbivores – tortoises. Within this group, a large range of body sizes can be obtained with minimal differences in digestive anatomy and physiology. The scaling of gut capacity with body mass is generally similar in herbivorous reptiles and mammals (Franz et al. 2009). The aim of our study was to test whether, in tortoises, voluntary food intake scales to metabolic body weight ($BM^{0.75}$), and methane production scales linearly with BM.

Materials and Methods

We performed intake and respiration chamber measurements in 24 individual tortoises of the species *Testudo graeca* (n=5, 1.16 ± 0.95 kg, range 0.52 – 2.83 kg), *T. hermanni* (n=6, 1.28 ±

0.36 kg, range 0.91 – 1.72 kg), *G. nigra* (n=2, 5.50 ± 0.28 kg, range 5.30 – 5.70 kg), *Geochelone sulcata* (n=8, 27.8 ± 18.0 kg, range 7.2 – 50.0 kg), *Dipsochelys dussumieri* (n=3, 141 ± 38 kg, range 104 – 180 kg). Animals were kept individually for 30 days at 27–30°C for intake measurements after an adaptation period of one week. The diet consisted of grass hay and salad in varying proportions. Water was available ad libitum at all times. Food offered and left over was quantified, and faeces were collected completely. Representative subsamples were used to determine dry matter (DM), crude protein, gross energy (GE) and neutral detergent fibre (NDF) concentrations using standard methods (AOAC 1997); these data allowed the calculation of the apparent digestibility of DM, GE and NDF (Robbins 1993). Experimental conditions or sample size did not always allow all analyses to be performed for all individuals (cf. Table 1). The ingested diets contained crude protein at 13.0 ± 1.8 %DM (range 9.5-17.0) and NDF at 48.8 ± 10.7 %DM (29.6-66.2) %DM.

At the end of the intake study, tortoises were transferred to open circuit respiration chambers constructed and operated as described in Soliva and Hess (2007) for two consecutive 22.5 h periods (temperature 29 ± 1 °C, constant humidity 60 %, pressure 987 ± 8 hPa; chambers for BM from 0.5-10 kg: volume 0.85 m^3, air flow 1.09 ± 0.08 m^3 h^{-1}; chambers for BM from 20-180 kg: volume 4.55m^3, air flow 6.08 ± 2.77 m^3 h^{-1}). Animals were measured individually except for the tortoises < 5 kg; after pilot measurements, two groups of five individuals between 0.5-2 kg and one group of three individuals between 2-3 kg were measured together, and results divided by the number of animals. All gas volumes were corrected for standard conditions (1013hPa, 0°C, 0% relative humidity). Following various conventions in the scientific literature, daily methane

production was not only expressed in absolute terms, but also in relation to DM, GE, digestible energy (DE) and digestible NDF (dNDF) intake. Data were analysed after ln-transformation using regression analysis with PSAW 18.0 (SPSS Inc., Chicago, IL), indicating 95% confidence intervals (*95%CI*) according to $y = a\ BM^b$.

Results

The data from tortoises showed a large amount of variance, often leading to large confidence intervals for the estimated parameters (Table 1); we observed a higher degree of variation in the food intake in the tortoises used than is common in feeding trials with mammals, even though we deliberately set the time period for the intake trials at 30 d. Mean daily DM intake (in kg) of the tortoises scaled to 0.005 (*95%CI* 0.004-0.007) $BM^{0.75\ (95\%CI\ 0.64\text{-}0.87)}$ (n=22, r^2=0.90, p<0.001) and mean GE intake (in kJ) scaled to 86.1 (*95%CI* 64.5-114.7) $BM^{0.77\ (95\%CI\ 0.66\text{-}0.88)}$ (n=21, r^2=0.92, p<0.001). In contrast, mean daily methane production scaled linearly to BM (Table 1, Fig. 1a). During measurements in the respiration chamber, it was noted that methane production was not constant throughout the day but occurred in distinct bursts (Fig. 2).

When expressed as energy loss in proportion of digestible energy intake or digestible NDF intake, methane losses scaled to $BM^{0.32}$ and $BM^{0.30}$, respectively (Table 1, Fig. 1bc). The *95%CI* overlapped for all factors *a* and exponents *b* between tortoises and nonruminant mammals (Table 1). Apart from the scaling exponent when methane was expressed per digestible energy intake (which was not significant in ruminants), the *95%CI* of the scaling exponent *b* also overlapped between tortoises and nonruminant mammals, and ruminants. In contrast, the *95%CI* of the factor *a* was invariably higher in ruminants than in the other two groups (Table 1).

Discussion

The results of this study suggest that in herbivores, methane production scales linearly with body mass, and the proportional losses due to methane output increase with increasing body mass. Although the existing data must still be considered scarce, the parallel findings in ruminant and nonruminant mammalian herbivores and herbivorous tortoises strongly suggest a general scaling pattern.

Similar scaling patterns in reptiles and mammals have been found for other parameters such as field metabolic rate (Bennett & Dawson 1976; Nagy et al. 1999), food intake (Meienberger et al. 1993; Clauss et al. 2007), or ingesta particle size (Fritz et al. 2010) – although on different levels; in turn, other measures appear to be relatively similar between herbivorous reptiles and mammals, such as the proportion of the gut contents of total body mass (Franz et al. 2009) or the achieved digestibilities (Karasov et al. 1986; Hatt et al. 2005). Generally, it is assumed that energy metabolism in reptiles is roughly a tenth of that observed in mammals (Kirkwood 1996). The difference in the intercept *a* of the regression equation describing dry matter intake in the tortoises of this study (0.005) compared to that found in herbivorous mammals in general (0.047 in Clauss et al. 2007) fits this pattern, as does the difference in the intercept describing the absolute methane output (0.014 in tortoises vs. 0.181 in nonruminant mammals, Table 1). Consequently, when methane production is expressed per unit intake, there is no significant difference in the intercept *a* between tortoises and nonruminant mammals (Table 1).

This finding is remarkable because it indicates a convergent adaptation of the gastrointestinal fauna between ectotherms and endotherms. Other similarities between the microbial faunas of herbivorous reptiles and mammals have been reported, such as the number of gut bacteria and the presence of protozoa (Fenchel et al. 1979; McBee & McBee 1982; Troyer 1984a), cellulase activity (Nagy 1977), or the concentration of fermentation products (Bjorndal 1979; Troyer 1984b; Foley et al. 1992; Barboza 1995). A relatively similar methane production per unit food intake in reptiles and mammals means that the processes of microbial fermentation must be similar even though the microbial faunas of reptiles and mammals will vary distinctively in their temperature sensitivity. The findings suggest that methane production is a more or less constant, unavoidable by-product of microbial fermentation in herbivores. Because of the well-documented differences in ingesta retention times between herbivorous reptiles (230 ± 140 h, reviewed in Hailey 1997) and mammals (40 ± 25 h, reviewed in Clauss et al. 2007), the similarity in methane scaling between reptiles and mammals also indicates that retention time as such is not the main influence factor for the scope of methane production, even if it may be relevant when comparing data within a species (Okine et al. 1989; Pinares-Patiño et al. 2003). Our results also suggest that the increase in methane production with increasing body size is not only due to an increase in fibre digestibility at higher body sizes; when experessed per unit of digestible fibre intake, the effect of an increasing methane production remains and scales similarly with BM as when expressed in relation to other intake measures (Table 1).

Prins and Kreulen (1991) and Van Soest (1994; p. 260) suggested that a different group of methanogenes – slower-growing archeae with a generation time of about 4 days that produce methane from acetate in sewers, for example – may actually limit body size in herbivores. They

considered ingesta retention a function of body mass (Demment & Van Soest 1985; Illius & Gordon 1992; but see Clauss et al. 2007) and hypothesized that when retention times surpass 4 days, energetic losses due to acetate-based methanogenesis would become prohibitve for the host. The fact that in herbivorous reptiles, retention times beyond 96 h are rather the rule than the exception (Hailey 1997; Hatt et al. 2002) indicates that other factors than retention time must limit the occurence of slow-growing archeae in herbivores.

An interesting question if is methane production by the fast-growing archeae should be considered a constraint on the evolution of body size. This has been suggested for ruminants, due to the high proportion of energetic losses due to methane in this group (Franz et al. 2010b); for nonruminant mammals, these losses might become limiting at extrapolated body masses of 100 metric tonnes (Franz et al. 2010a). If the regression equation from tortoises is applied to the largest known chelonian, *Archelon ischyros*, a marine turtle with an estimated maximum BM of 5000 kg (Anonymous 1999), energetic losses due to methane of nearly 14 % of digetible energy intake are extrapolated, which is similar to losses found in extant large ruminants. Note that this similarity to ruminants, in spite of the general similarity in scaling between tortoises and nonruminant mammals, is due to the determined exponent *b* of 0.32, which is numerically higher than the one calculated for nonruminant mammals (0.17), though overlapping in its confidence interval. Differences in exponent should be considered with caution when extrapolations beyond the BM range are performed that served to generate the regression equation (Franz et al. 2009).

One last interesting question is why herbivores apparently did not evolve to avoid methane losses. Intervention studies in domestic ruminants showed that in principle, a functional

fermentation chamber can be maintained in the absence or near-absence of archeae and according methane production (e.g. Sawyer et al. 1974; McCrabb et al. 1997; Goel et al. 2009; Tomkins et al. 2009). An alternative view at methanogenes could be that they are among the prerequisites for herbivory: Pimentel et al. (2006) showed that, in a combined model with dogs and guinea pigs, methane slowed intestinal passage by decreasing intestinal contractile activity. While offering new insights into potential therapeutical interventions against human irritable bowel syndrome, these results also give rise to the speculation that the presence of methane, and its passage-delaying effect, was an important component of the evolution of physiological adaptations to herbivory (which requires long passage times). However, this hypothesis requires much further research.

To conclude, our study shows that methane losses occur not only in mammalian but also in reptilian herbivores and that they scale linearly with body mass, thus representing proportionally increasing losses at increasing body size. Further studies combining in vivo measurements and microbiological analyses should unravel the fundamental principles behind the link between microbial fibre fermentation in vertebrate herbivores and methane production.

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Table1. Allometric scaling relationships for tortoises, mammalian nonruminants and ruminants with body mass (BM) according to the equation $y = a\ BM^b$.

Herbivore group	*y*	unit	n*	*a*	*95% CI a*	*b*	*95% CI b*	r^2	*p*
Tortoises	Methane	$L\ d^{-1}$	24	0.014	0.009-0.023	1.03	0.84-1.22	0.85	<0.001
Nonruminants			41	0.181	0.144-0.227	0.97	0.92-1.02	0.98	<0.001
Ruminants			62	0.661	0.420-1.040	0.97	0.88-1.07	0.87	<0.001
Tortoises		$L\ (kg\ DMI)^{-1}$	22	3.02	2.07-4.40	0.33	0.18-0.47	0.52	<0.001
Nonruminants			25	3.34	2.63-4.26	0.16	0.10-0.22	0.59	<0.001
Ruminants			45	16.58	12.17-22.60	0.12	0.06-0.18	0.25	<0.001
Tortoises		$L\ (kJ\ GEI)^{-1}$	21	0.70	0.47-1.05	0.29	0.139-0.446	0.46	0.001
Nonruminants			25	0.79	0.63-0.99	0.15	0.093-0.204	0.57	<0.001
Ruminants			44	3.53	2.52-4.94	0.13	0.058-0.195	0.25	<0.001
Tortoises		$L\ (kJ\ DEI)^{-1}$	16	0.91	0.51-1.60	0.32	0.13-0.51	0.45	0.003
Nonruminants			31	1.48	1.21-1.81	0.17	0.13-0.21	0.71	<0.001
Ruminants			35	7.87	5.13-12.06	0.09	-0.001 – 0.18	0.11	0.053
Tortoises		$L\ (g\ dNDFI)^{-1}$	21	10.1	6.6-15.5	0.30	0.13-0.46	0.43	0.001
Nonruminants			23	11.1	9.1-13.5	0.17	0.12-0.22	0.70	<0.001
Ruminants			17	57.4	26.3-125.2	0.11	-0.05 – 0.27	0.12	0.170

DM dry matter, GE gross energy, DE digestible energy, dNDF digestible neutral detergent fibre, I intake

tortoise data from this study; ruminant data collection from Franz et al. (2010b), nonruminant data collection from Franz et al. (2010a)

*sample sizes vary between measurements because for tortoises, not all measurements could be performed because of logistic reasons, and because for mammals, data available from the literature varied between sources

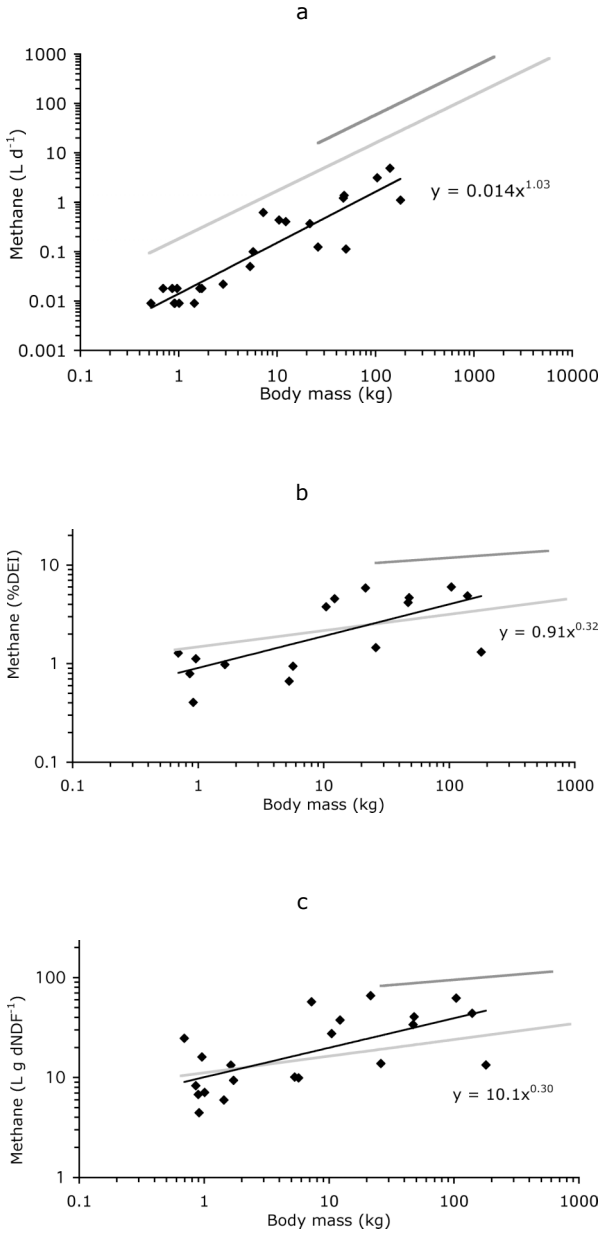

Figure 1. Relationship between body mass and a) absolute daily methane production, b) methane energy losses in % of daily digestible energy intake and c) methane energy losses related to the daily intake of digestible cell wall (neutral detergent fibre). Data for ruminants (dark grey regression line; data collection from Franz et al. 2010b), nonruminant mammalian herbivores (light grey regression line; data collection from Franz et al. 2010a) and tortoises of this study.

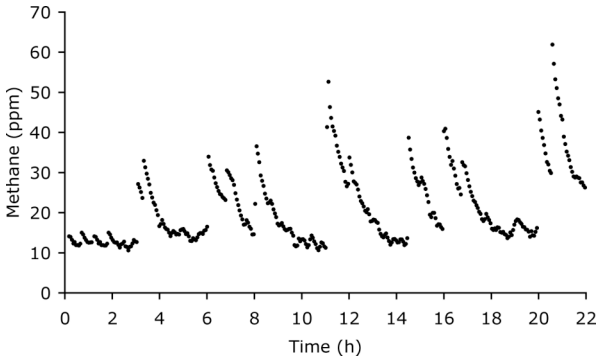

Figure 2. Example of methane measurements in an open circuit respiration chamber in a *Geochelone sulcata* (10.5 kg) for one uninterrupted measurement period of 22 hours.

Appendix

Scientific names of species used in this thesis

Mammalia

Artiodactyla

Sheep	*Ovis aries*

Lagomorpha

Rabbit	*Oryctolagus cuniculus*

Rodentia

Guinea pig	*Cavia porcellus*

Perissodactyla

Mini Shetland pony	*Equus ferus caballus*

Reptilia

Testudinata

African spurred tortoise	*Geochelone sulcata*
Galapagos tortoise	*Geochelone nigra*
Giant Aldabra tortoise	*Dipsochelys dussumieri*
Greek tortoise	*Testudo hermanni*
Hermann's tortoise	*Testudo graeca*

Respiratory chamber

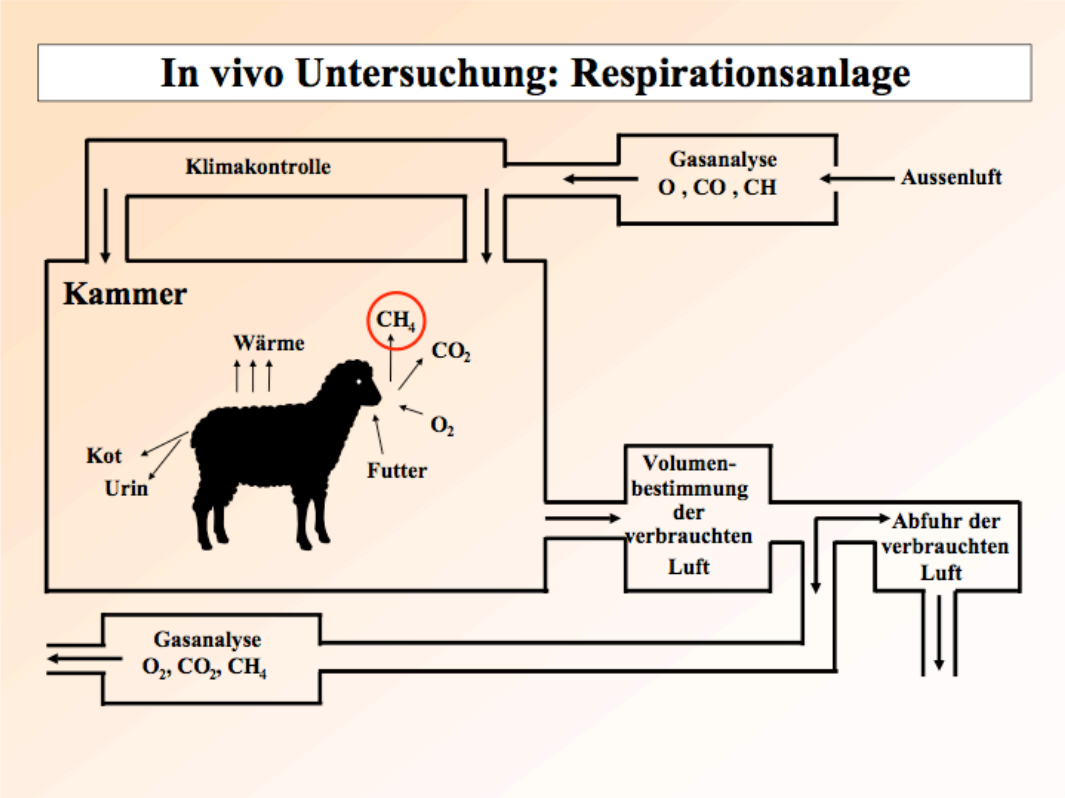

Table 1: Exemplarily, an outline of a respiratory chamber used in the experiments (C. R. Soliva & H.D. Hess 2007 Measuring methane emission of ruminants by in vitro and in vivo techniques. In *Measuring methane production from ruminants* (ed. H. P. S. Makkar & P. E. Vercoe). Dordrecht: Springer).

No 'walking compost heaps': Core body temperature fluctuations in Giant Aldabra tortoises *(Dipsochelys dussumieri)* do not suggest relevant contribution of fermentation to body heat

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Running head: Tortoise body temperature

Summary

1. It is thought that large reptiles and dinosaurs achieve relatively constant body temperatures (T_b) due to thermal inertia, and that in herbivores, fermentative heat generated by intestinal microbes will contribute to a stable T_b.

2. We measured ambient (T_a) and T_b fluctuation in three captive Giant Aldabra tortoises (*Dipsochelys dussumieri*; 100-180 kg) by feeding temperature probes to the animals (recording time 8-11 days).

3. T_b varied by 9°C between daily minima and maxima, and clearly fluctuated with T_a; the dependence on ambient temperatures was particularly evident during the single cloudy day of the observation period.

4. When adding this data to a literature compilation of data on body mass, T_a range and T_b range, there was no effect of feeding type (carnivore vs. herbivore) on ΔT_b, indicating no additional benefit due to intestinal fermentation.

5. Additionally, no effect of body mass on ΔT_b was evident when ΔT_a was controlled for.

6. The result suggest that ectotherm herbivores are unlikely to benefit from intestinal fermentation in terms of thermal stability, and that inertial homoiothermy most likely is relevant at body masses above that of extant herbivorous reptiles.

Key words: ectotherm, inertial homoiotherm, digestion, microbial fermentation, herbivore, thermal physiology

Introduction

In ectotherms body temperature (T_b) is mainly a consequence of, and is consequential to, their physiological ecology (Zimmerman, Connor, Bulova *et al.*, 1994, Tosini & Menaker, 1995) and oscillates often with the thermal fluctuation of the environment (McNab & Auffenberg, 1976, Rummery, Shine, Houston *et al.*, 1994). While some species seem to be thermally passive (T_b driven only by fluctuation of the ambient temperature (T_a)), others can actively maintain their T_b, albeit within limits determined by environmental conditions (Troyer, 1987).

Active T_b regulation in ectotherms includes behavioural mechanisms, like selection of different ambient temperatures, basking in the sun or maximizing sun exposure. Therefore, air temperature alone often does not adequately reflect the thermal variables to which animals are exposed. Especially when basking in sunny places, ectotherms can attain much higher body temperatures than the ambient temperature (Troyer, 1987). Furthermore, T_b in ectotherms can increase as consequence of physiological performance, like locomotion and prey capture (Lailvaux & Irschick, 2007, Van Damme, Bauwens & Verheyen, 1991). In addition, T_b in *Iguana iguana* has been demonstrated to display physiologically generated circadian rhythms in a constant environmental temperature, similar to those recorded in endotherms (Tosini *et al.*, 1995). Huot-Daubremont et al. (1996) even reported the endogenous production of significant amounts of heat in hibernating Hermann's tortoises (*Testudo hermanni*).

One of the most important factors in thermal biology is body size. The body surface influences the rate at which heat is exchanged with the environment. Due to a favourable surface-volume ratio, larger ectotherms should be less responsive to their thermal environment than smaller ones

(Zimmerman & Tracy, 1989). For example, Seebacher (2003) compared T_b fluctuations in crocodiles ranging from 2.5 to 1010 kg, and showed a decrease with increasing body mass; at the same time, mean T_b itself increased with increasing body mass. Stevenson (1985b) presented a heat balance model to quantify the effects of body size on T_b in ectotherms and concluded that reptiles larger than 1 kg cannot heat or cool quickly enough to follow the daily cycling of the thermal environment precisely. For that reason, the range of T_b that these reptiles can experience should be less than the range of ambient temperatures to which they are exposed. Body size affects the rate of heat absorption from the sun (Brattstorm, 1965), as larger bodies display a higher absorption area. Larger reptiles have a lower thermal conductance related to small surface-to-volume ratio and thick integuments, which leads to a notable heat storage capacity (McNab *et al.*, 1976). Furthermore McNab & Auffenberg (1976) concluded that the greater the body size, the smaller the cooling constant, and the longer it would take to attain a thermal equilibrium with the environment. This would result in similar temperature differentials between T_b and T_a in reptiles and mammals at weights greater than 100 kg. An important data point included in these considerations is one measured by Mackay (1964) in a 170-kg Galapagos tortoise (*Geochelone nigra*), which had a relatively small daily T_b variation of only 4.3°C.

Extant reptilian, mammalian and avian herbivores, as well as probably herbivorous dinosaurs, rely on symbiotic gut microflora to digest plant fibre. In all digestive processes heat is dissipated, whether due to alloenzymatic (microbial digestion) or autoenzymatic (host's own) digestion. A certain amount of heat is always produced during microbial growth due to the exothermic nature of substrate energy recovery (Luong & Volesky, 1982). Heat production as a byproduct of fermentation in the gastrointestinal tract of ruminants has been the focus of several *in vitro* and

in vivo studies (Webster, Osuji, White *et al.*, 1975, Marston, 1948, Houpt, 1968). Active microbial populations in large fermentation chambers have been hypothesized to contribute significantly to temperature regulation. Marston (1948) postulated, that energy, dissipated as heat in the metabolic processes of microorganisms, could be an advantage for animals living under cold conditions; according to this author, this heat might be used to maintain body temperature without wasting combustible energy of nutrients. Because of the extrapolated large volume of fermenting ingesta in the guts of the biggest terrestrial herbivores, the sauropods (Franz, Hummel, Kienzle *et al.*, 2009), Farlow (1987) compared those fermentation chambers to giant compost heaps that might have been a significant source of thermoregulatory heat. Of course, this is rather an allegory than a real comparison, because composting is a process based on aerobic microbial digestion, in contrast to the anaerobic fermentation occurring in the gut of herbivores. The typical 'heat' associated with compost heaps is a result of the self-isolating nature of these heaps that allows a temperature accumulation. However, given the exothermic nature of metabolic or digestive processes (either in aut-enzymatic digestion in carnivores or allo-enzymatic digestion in herbivores), herbivores might generally have an advantage in endogenous heat generation due to their higher and more continuous food intake (due to the lower digestibility of their food) as compared to carnivores. In other words, the advantage would not be that they harbour fermenting microbes in their gut, but that more digestion and fermentation has to take place, i.e. more metabolic steps, before the same amount of energy is available for the host organism as compared to carnivore digestion.

However, digestion itself is temperature dependent in carnivorous as well as herbivorous reptiles (reviewed by Zimmerman *et al.*, 1989), with lower efficiency at low temperatures. Thus, rather

than generating heat, digestion in herbivorous reptiles might rather require a certain ambient temperature for optimal function. In order to test whether microbial fermentation and large body size lead to a comparatively stable core body temperature, as suggested by the single experiment by Mackay (1964), we determined core body temperatures in three individuals of one of the largest extant reptilian herbivores – Giant Aldabra tortoises (*Dipsochelys dussumieri*).

Materials and Methods

Three adult Giant Aldabra tortoises (40-70years; 1 female: 104kg and 2 males: 140kg and 180kg) from Zurich Zoo were used for this study. The tortoises were housed in a compartment within a 11000m^2 indoor enclosure, the Masoala Rainforest exhibit (Bauert, Furrer, Zingg *et al.*, 2007). Data were obtained during summer 2009. In the course of the experiment, the animals had *ad libitum* access to drinking water, food (grass hay, freshly cut grass, salad), and both shade and direct sunlight. Environmental temperatures were recorded every 15 minutes for the whole experiment period by placing HC temperature/humidity Loggers (OnSolution Pty Ltd., Baulkham Hills 2153, Australia) at three different locations (shaded, under a tree; directly exposed to sunlight; shaded, over stony ground), preferred by the animals (height between 0.3-1.0m). For some comparisons, these three measurements were averaged as one 'environment reading'. Additionally, temperatures were directly measured and documented on the loggers by a temperature pistol (Raytek Fluke 566, Raytek Corporation, Santa Cruz, USA) to test whether logger and pistol data could be reliably compared without systematic deviation. Logger and pistol measurements were highly correlated ($R=0.754$, $p<0.0001$, $n=78$), with no significant difference between the two (paired t-test $p=0.214$). Core BT was recorded every 15 minutes with similar temperature-sensitive devices (HC temperature/humidity Logger), which were swallowed

by the tortoises. Faeces were continuously examined for the ingested loggers, which were recovered 8-11 days after ingestion. Data were downloaded from all loggers using eTemperature software (Version 5.10; OnSolution Pty Ltd., Baulkham Hills 2153, Australia). Additionally, surface temperatures of the carapace (measuring temperature in the centre of each of the 13 main scutes, and averaging these measurements as one 'carapace reading'), the extremities (measuring temperatures on each extremity in the region of the metacarpal/metatarsal joints, and averaging these measurements as one 'extremity reading'), and the deep skin folds (measuring surface temperature of the skin at the deepest point underneath the carapace between each fore extremity and the neck, and between each hind extremity and the tail, and averaging these four measurements as one 'skin fold reading') were documented regularly at 1-3 h intervals using the Raytek Fluke 566 temperature pistol for a period of 48h during days 5 and 6 of the experiment.

To put our results into a comparative context, we collated data on the range of T_b, T_a and body mass in reptiles from the scientific literature (see Fig. 3 for a list of sources). For two studies, body masses of species had to be estimated (McGinnis & Dickson, 1967, Troyer, 1987). When the feeding type was not mentioned in the study itself, it was consulted in (Grzimek, 1980). Body mass data was ln-transformed. In order to test whether the existing data allowed conclusions on the influence factors for the range in T_b, we used correlation analysis and a General Linear Model in SPSS 18.0 (SPSS Inc., Chicago, IL), with T_b range as the dependent variable, T_a range and body mass as covariates, and the feeding type (herbivore, i.e. assuming microbial fermentation, vs. carnivore/omnivore); because body mass was highly correlated with the T_a range (see results), indicating that observations on larger animals had been made generally with lower T_a fluctuations, we included the body mass – T_a range interaction in the model. The

significance level was set to 0.05. It should be noted that due to the uneven distribution of species in the dataset, which included multiple measurements on individual species if available, and due to the different methods employed in the various study to define T_a, this approach should be considered explorative.

Results

The environmental temperatures measured at three different locations had similar daily minima but differed distinctively in their respective maxima (Table 1). Core temperatures recorded in the tortoises followed an identical pattern in all animals that was tightly linked to the environmental temperature (Fig. 1). During one cloudy day with a lesser peak in environmental temperatures and less opportunity for basking, the core temperature of all animals dropped markedly below the level achieved during the other days (Fig. 1, Table 1).

Daily temperature fluctuations were larger on the carapace surface than on the surface of the extremities, where in turn it was larger than in the inner skin fold or the core gut temperature (Fig. 2, Table 2). Turning points of the temperature curve occurred first in the environment, followed by the carapace, the extremities, the skin fold and finally the core (Fig. 2). The magnitude of the temperature range decreased from the smallest to the largest animal (Table 2); whereas the difference in range between the smallest and the largest animal was 1.2 °C for the carapace, 1.0 °C for the extremities and 0.9 °C for the skin fold, it was distinctively higher at 2.0 °C for the core temperature. All measured temperatures, including those of the environment, showed a steeper daily increase and a shallower daily decrease (Fig. 2).

When plotting the observed T_b range against body mass, following an approach by Stevenson (1985a), there seemed to be a correlation that indicates that with increasing body mass, reptiles have a lower fluctuation in T_b (Fig. 3a; Pearson's R=-0.642, p<0.0001, n=44). However, when plotting the range of T_b from the literature and this study against the range of T_a observed in the respective studies, it is evident that the variation in T_b followed that in T_a (Fig. 3b; Pearson's R=0.814, p<0.0001, n=44). Actually, the range of T_a observed in the studies was highly correlated to the body mass of the animals that were studied (Pearson's R=-0.402, p=0.007, n=44). When the range of T_b was expressed as a ratio of T_a, the correlation of this quotient with body mass was significant (Pearson's R=-0.482, p=0.001, n=44), although the according plot was not convincing (Fig. 3c). Actually, if crocodiles were excluded from the dataset, no such correlation was evident (Pearson's R=-0.198, p=0.241, n=37). The General Linear Model for the whole dataset (R^2=0.795, F=31.097, p<0.0001, n=44) showed that while the range of T_a had a significant influence on the range of T_b (p<0.0001), neither feeding type (p=0.335) nor body mass (p=0.840) had a significant influence on the range of T_b (with the interaction term of T_a range and body mass tending towards significance at p=0.095).

Discussion

In the recent study, T_b of three giant tortoises (100-180kg) were recorded, which varied by about 9°C. Our results are in contrast to data documented by Mackay (1964), in a single Galapagos giant tortoise (170kg), with a relatively small core temperature range of 4°C. Compared with our data, Mackay (1964) also documented a lower range of ambient temperatures, which will most likely have led to the seemingly more stable T_b. As the time intervals of temperature readings were, with 1-4 h, larger in the previous experiment than in the one presented here (15 min), it is

theoretically possible that Mackay's (1964) measurements missed the maximum and minimum peaks of body temperature of his animal, which might be another reason for the lower T_b range.

The core temperature of the tortoises, though following fluctuations of the environment, was always higher than the environmental temperatures measured in the shade; only the temperature logger placed in direct sunlight recorded temperatures that were, especially during the morning part of the day, higher than the tortoises' core temperature (Fig. 1). The temperatures measured on the surface of the carapaces were notably higher than ambient temperature, which is evidence for basking behaviour. While basking in the sun, ectotherms reach higher core temperatures than air temperatures, and T_b correlates positively with time spent basking (Troyer, 1987, Stebbins & Barwick, 1968). Correspondingly, core temperatures dropped in our tortoises during the one cloudy day where basking was not possible (Fig. 1). This response is in agreement with previous studies, which have shown circadian daily rhythms in reptiles (Troyer, 1987, Mackay, 1968, Stebbins *et al.*, 1968). A comparison of reptilian T_b showed that T_b of desert tortoises were more variable than in other reptiles (Zimmerman et al., 1994), most likely due to the enormous range in environmental temperature and direct sun radiation to which desert animals are subjected.

Body temperatures of reptiles have been measured by a wide variety of methods. Benedict (1932) compared temperatures measured on the skin of the neck and the groin with rectal temperatures of South American gopher tortoises (*Testudo denticulata*) and got results which were not significantly different. In the present study, skin (extremities and between extremities/neck or tail) and core temperatures (measured in the gastrointestinal tract) were determined, and core temperatures were distinctively higher than those measured on the skin of

the tortoises (Table 2). These results imply that in large reptiles, differences between cloacal and core temperatures do exist; even though both terms are often used equally as "body temperature". In other studies repeated individual measurements, which require regular handling (rectal) (Brattstorm, 1965, Troyer, 1987), were performed. In some former studies, body temperature were measured by in freshly shot reptiles (Bogert, 1949, Inger, 1959). Temperature probes have been attached on the outside of an animal (Zimmerman *et al.*, 1989, Christian, Tracy & Porter, 1983), applied internally by surgery (Mackay, 1968, Stebbins *et al.*, 1968) or by feeding (Mackay, 1964, Lutterschmidt & Reinert, 1990). The key benefits of feeding transmitters are evident: body temperatures can be recorded throughout the day without any relevant disturbance of the animal. However, for a reliable investigation of the thermal physiology of ectotherms, not only the body temperature, but also the ambient temperature must be recorded in a reliable way. Brattstrom (1965) summarized problems in temperature measurements: possible effects of basking, heat conduction from the substrate, and retention of heat from previous basking behaviour have often been overlooked or not mentioned. Furthermore common sources of error in obtaining T_b are e.g. the heat exchange from warm hands of the collector to the cold body of the ectotherm, or unrecorded artificial heat sources in a terrarium that will not go on record in air temperature measured at a distance from the heat source. Other researchers recorded temperatures of black bulbs of different sizes and shapes in the same environment as the examined animals, in order to measure the heat of solar absorption rather than ambient air temperatures (Bartholomew, 1966, Vitt & Sartorius, 1999, Grant, 1990). So far, larger data collections such as the one presented in Fig. 3 must be considered exploratory because of the unstandardized way that body temperatures, but even more so ambient temperatures are recorded.

Body size is usually considered a crucial factor in thermal biology. Larger ectotherms are thought to be less responsive to their thermal environment than smaller ectotherms (Spotila, Lommen, Bakken *et al.*, 1973, Colbert, Cowles & Bogert, 1946), and this characteristic is often referred to as "inertial homeothermy" (McNab *et al.*, 1976). While the theoretical basis for this concept is valid, the empirical data by which it can be tested across a range of species does not support this concept (Fig. 3c). Field data of crocodiles showed that body temperature fluctuations decrease with increasing body mass (Grigg, Seebacher, Beard *et al.*, 1998, Seebacher, 2003), but for other reptile groups or species, similar comparative measurements under identical field conditions, or under standardized laboratory conditions, are lacking. The comparatively large fluctuations in the tortoises in this study, as well as the exploratory statistics performed on the overall dataset, appear to indicate that within the body size range of extant reptiles, large ectotherms are subject to similar fluctuations in body temperature as smaller ones once variation in ambient temperature has been taken into account (Fig. 3c). These findings support the conclusion of Grigg et al. (2004) that inertial homeothermy over the course of a single day will only be found in large ectotherms above 500 kg of body size. Therefore, tests of thermal inertia in non-crocodilian ectotherms – remember that crocodiles are considered to be secondary ectotherms (Seymour, Bennett-Stamper, Johnston *et al.*, 2004) - would have to be performed in fish, as no other extant terrestrial ectotherm surpasses this threshold.

The patterns displayed in Fig. 3, as well as the explorative statistics on the overall dataset, do not suggest that feeding type, and hence the presence or absence of microbial fermentation in the digestive tract, has an influence on the thermal physiology of reptiles. Even in the

gastrointestinal tract of African elephants (*Loxodonta africana*) – the largest terrestrial herbivores with an enormous gut capacity (Clauss, Robert, Walzer *et al.*, 2005) - a daily fluctuation of gut content temperature of 1°C can be observed (Kinahan, Inge-moller, Bateman *et al.*, 2007). Although these findings cannot prove the absence of a contribution of intestinal fermentation to overall body temperatures, they do not indicate that this fermentation maintains a constant thermal intestinal environment. Instead, in herbivorous reptiles, digestive processes vary markedly with environmental (and hence body) temperature (Zimmerman *et al.*, 1989), rather than being decoupled from the environment. In mammals, a comparison of body temperatures across a large variety of mammalian species led to the conclusion that the contribution of fermentation heat to overall temperature regulation, if it exists at all, does not follow a consistent pattern (Clarke & Rothery, 2008). Taken together, these observations indicate that intestinal fermentation is not a relevant source of heat production, and is unlikely to have contributed to homoiothermy in dinosaur herbivores.

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Table 1. Ambient and body temperatures (°C) for three Giant Aldabra tortoises *(Dipsochelys dussumieri)* of different body mass.

	Days 2-6, 8-12	Day 7*
Enivronment 1 (shade, stone ground)	17.6-33.1	17.6-26.1
Environment 2 (exposed to sun)	18.2-36.7	18.2-31.7
Environment 3 (shade, under tree)	17.1-28.6	17.1-24.1
Core (100 kg)	26.6-34.1	25.1-27.6
Core (140 kg)	26.7-34.2	24.7-27.2
Core (180 kg)	26.7-33.2	24.2-26.7

*the only particularly cloudy day during the experimental period

Table 2. Body temperatures (°C) for three Giant Aldabra tortoises *(Dipsochelys dussumieri)* of different body mass*.

	Carapace	Extremities	Skin fold	Core
100 kg	21.0-40.4	23.7-35.1	25.6-33.2	26.1-34.1
140 kg	21.1-40.5	23.8-35.0	25.8-32.6	26.2-33.7
180 kg	21.6-39.8	23.6-34.1	25.3-32.0	26.2-32.2

*ambient temperature ranges: 1 – 18.1-32.1; 2 – 18.2-36.6; 3 – 17.6-28.6

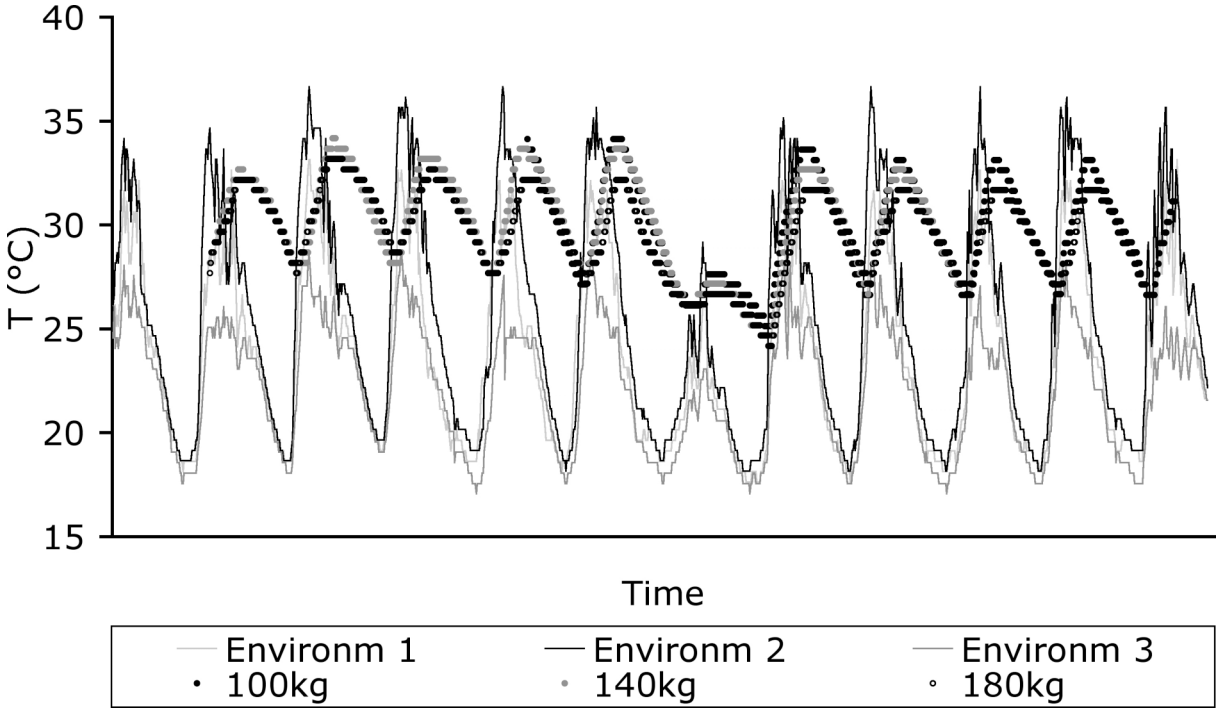


Figure 1. Fluctuation of environmental (measured at three different locations in the enclosure) and core (gastrointestinal) temperature of three giant tortoises *(Dipsochelys dussumieri*) of different body mass over a course of 12 days. Note the uniformity of the overall pattern, the generally lower peaks of the core temperature of the largest animal, and the decrease of core temperature in the course of a colder day (day 7).

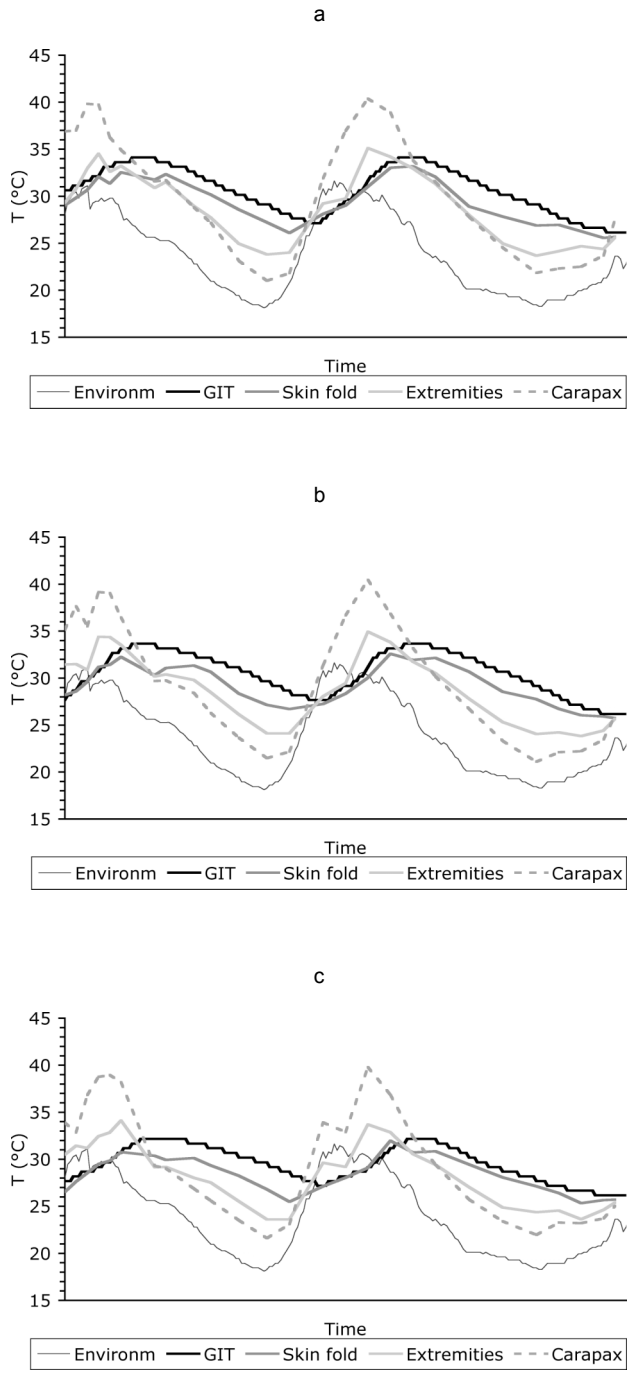
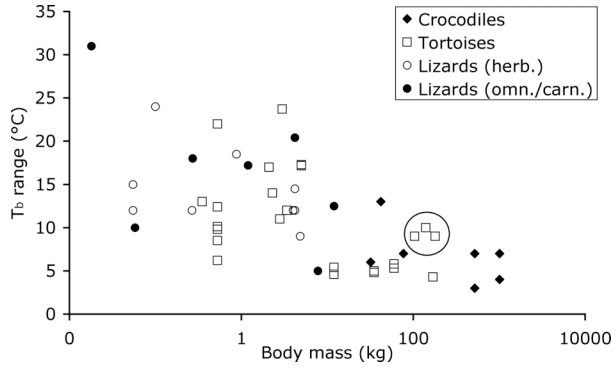
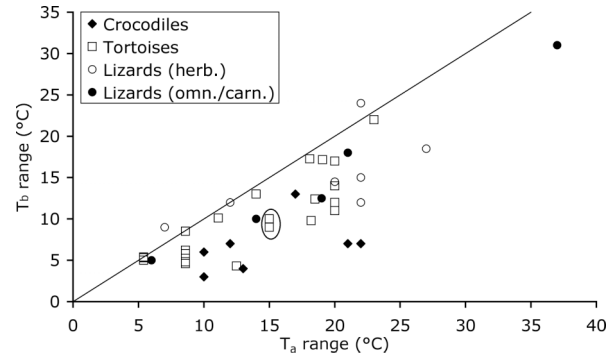


Figure 2. Fluctuations of average environmental temperature, core (GIT) temperature, and the surface temperature of the carapace, the extremities, and the inner skin fold in three giant tortoises *(Dipsochelys dussumieri)* of a) 100 kg, b) 140 kg, c) 180 kg.

a


Crocodiles
Tortoises
Lizards (herb.)
Lizards (omn./carn.)
Tb range (°C)
0
5
10
15
20
25
30
35
0
1
100
10000
Body mass (kg)

b


Crocodiles
Tortoises
Lizards (herb.)
Lizards (omn./carn.)
Tb range (°C)
0
5
10
15
20
25
30
35
0
5
10
15
20
25
30
35
40
Ta range (°C)

c

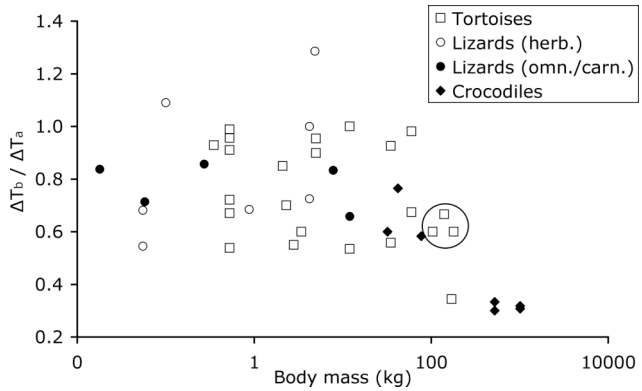

Tortoises
Lizards (herb.)
Lizards (omn./carn.)
Crocodiles
ΔTb / ΔTa
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
1
100
10000
Body mass (kg)

Figure 3. Relationship between a) body mass and documented daily variation in core temperature in reptiles using raw data for body temperature (following Stevenson, 1985a), indicating a decrease in temperature variation with body mass (Thompson, Pianka & de Boer, 1998, Wikramanayake & Green, 1989, Christian, Clavijo, Cordero-Lopez *et al.*, 1986, McGinnis *et al.*, 1967, Throckmorton, 1973, Marlow, 1979, Pulford, Hailey & Stubbs, 1984, Huot-Daubremont *et al.*, 1996, Zimmerman *et al.*, 1994, Swingland & Frazier, 1979, Mackay, 1964, Case, 1976, Troyer, 1987, Christian *et al.*, 1983, Pearson & Bradford, 1976, Sokolov, Sukhov & Chernyshov, 1975, Stebbins *et al.*, 1968, Rummery *et al.*, 1994, McNab *et al.*, 1976, Grigg *et al.*, 1998, Benedict, 1932) b) plotting the variation in body temperature (T_b) vs. ambient temperature (T_a) fluctuation, indication that T_b follows T_a at a systematically lower level (line: y=x) (same data as in 3a expect *) and c) plotting the quotient of variation in T_b per variation in T_a vs. body mass, indicating that there is little systematic decrease of the quotient with body mass (same data as in 3a expect *). Ambient temperature is not defined consistently throughout studies. Data from this study (three Giant Aldabra tortoises) indicated by circle. The low value in 3c is the Galapagos tortoise from Mackay (1964). Note that there is no systematic difference between herbivorous and carnivorous reptiles.

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Intake, selection, digesta retention, digestion and gut fill of two coprophageous species, rabbits (*Oryctolagus cuniculus*) and guinea pigs (*Cavia porcellus*), on a hay-only diet

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Keywords:

digestion, herbivory, hindgut fermenter, caecum fermenter, coprophagy, cecotrophy

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Summary

A colonic separation mechanism (CSM) is the prerequisite for the digestive strategy of coprophagy. Two different CSM are known in small herbivores, the 'wash-back' CSM of lagomorphs and the 'mucous-trap' CSM of rodents. Differences between these groups in their digestive pattern when fed exclusively hay were investigated in six rabbits (*Oryctolagus cuniculus*) and six guinea pigs (*Cavia porcellus*). Intake, digestibility (by total faecal collection), solute and particle mean retention times (MRT, using Co-EDTA and Cr-mordanted fibres) were measured. Rabbits selected less fibrous parts of the hay than guinea pigs, leaving orts with higher content of neutral detergent fibre (NDF; 721 ± 21 vs. 642 ± 31 g/kg dry matter (DM) in guinea pigs). They also expressed a lower NDF digestibility (0.44 ± 0.10 vs. 0.55 ± 0.05 of total), a similar particle MRT (15 ± 3 vs. 18 ± 6 h), a longer solute MRT (51 ± 9 vs. 16 ± 4 h), and a lower calculated gut fill (19.6 ± 4.7 vs. 29.7 ± 4.1 g DM/kg body mass) than guinea pigs ($p < 0.05$ for each variable). These results support the assumption that the 'wash-back' CSM, exhibited in the rabbits, is more efficient in extracting bacterial matter from the colonic digesta plug than the 'mucous-trap' CSM found in the guinea pigs. Related to metabolic body mass, rabbits therefore need a less capacious colon for their CSM where a more efficient bacteria wash-out is reflected in the lower fibre digestibility. A lighter digestive tract could contribute to a peculiarity of lagomorphs: their ability to run faster than other similar-sized mammals.

Introduction

In many small mammalian herbivores – mainly represented by lagomorphs (rabbits, hares and pikas) and rodents – the practice of coprophagy has been documented (Kenagy and Hoyt, 1980; Hirakawa, 2001, 2002). Actually, it was suggested that this digestive strategy should be assumed to occur in any lagomorph or herbivorous rodent until the opposite is proven (Clauss et al., 2007a). Coprophagy ensures that protein synthesised by bacteria growing in the distal fermentation chambers, the caecum and the colon, is not lost via defaecation but reingested. Additionally, other bacterial products like vitamins or undigested essential nutrients like fatty acids are used by the herbivore in this way (Karasov and Martínez del Rio, 2007; Leiber et al., 2008). Coprophagy appears to occur only in small herbivores, with the largest known coprophageous animal being the largest rodent, the capybara (*Hydrochaeris hydrochaeris*) (Hirakawa, 2002). One reason for this association with size may be that small herbivores cannot compensate for metabolic losses on low-quality forage by using body reserves, and thus have to maintain high food intakes on low-quality forages and minimize metabolic losses via coprophagy (Meyer et al., 2010).

A prerequisite for the practice of coprophagy is a mechanism in the digestive tract that separates the valuable material (mainly bacteria and small particles) from indigestible or hardly digestible residues, i.e. a 'colonic separation mechanism' (CSM) (Björnhag, 1987). Basically, two types of CSM exist (Cork et al., 1999): a 'wash-back' CSM as found in lagomorphs, and a 'mucus-trap' CSM as found in rodents. The colon of lagomorphs is characterised by three taenia and haustrae in the first, and one taenia with haustrae in the second part of the proximal colon; fluid secretion and retrograde peristalsis occurs during the phase when hard faeces are formed (Clauss, 1978; Snipes et al., 1982; Ehrlein et al., 1983). Thus, fluids, bacteria and small particles

are washed back into the caecum. Different from that, the colon of caviomorph and hystricomorph rodents is equipped with a peculiar anatomical structure, the 'colonic groove' or 'furrow' (Gorgas, 1966; Snipes et al., 1988). In this groove, mucous and bacteria are trapped and transported back to the caecum (Holtenius and Björnhag, 1985; Takahashi and Sakaguchi, 2000, 2006). The colon of some myomorph rodents is characterised by anatomical structures like longitudinal folds and oblique furrows (*Plicae circulares*) that may serve a similar purpose as the colonic groove in caviomorph rodents (Behmann, 1973; Sperber et al., 1983). The CSM type can be differentiated by the use of passage markers (Cork et al., 1999; Pei et al., 2001): The 'wash-back' CSM is characterised by short particle but long fluid retention times, whereas the 'mucus trap' CSM results in a more or less simultaneous excretion of fluid and particle passage markers.

The question whether the two CSMs differ in more than the fluid retention pattern has been hardly addressed (Björnhag and Snipes, 1999). Discussions of this topic focus mainly on the appearance of the faeces. In lagomorphs, two different types of faeces are formed: the so-called 'hard' faeces, which are mostly not re-ingested and which consist of larger particles, and the so-called 'soft' faeces or 'caecotrophs' that are re-ingested (Hirakawa, 2001). In contrast, such a separation of faeces types is considered less evident in rodents (Björnhag and Snipes, 1999; Hirakawa, 2001). However, different types of faeces were also described for beavers (*Aplodontia rufa*) (Hirakawa, 2001) and nutria (*Myocastor coypus*) (Takahashi and Sakaguchi, 1998), guinea pigs (Holtenius and Björnhag, 1985), capybaras (Mendes et al., 2000), dassie-rats (*Pteromus typicus*) (Mess and Ade, 2005), and tuco-tucos (*Ctenomys talarum*) (Martino et al., 2007). Nevertheless, less easily identifiable cecotrophs in rodents are a reason why the CSM of lagomorphs is considered more efficient than that of rodents (Björnhag and Snipes, 1999).

Differences between the CSM, other than those in fluid passage and visual appearance of the caecotrophs, have not been addressed so far.

The objective of the present study was, therefore, to compare diet selection, digesta retention, digestibility and calculated gut capacity in rabbits and guinea pigs as representatives for lagomorphs and rodents, respectively. Although a direct comparison of the two species has been published previously (Sakaguchi et al., 1987; 1992), this was done using a complete and pelleted feed. In contrast, we compared the species on a hay-only diet reflecting more their natural diet.

Materials and methods

Six pygmy rabbits (1.57 ± 0.31 kg) and six guinea pigs (0.79 ± 0.07 kg) were housed individually at 20 ± 2 °C on a 12 h light : 12 h dark schedule in cages (55 x 53 x 60 cm for guinea pigs and 97 x 60 x 55 cm for rabbits) with a carton-covered floor. Coprophagy was not prevented, or accounted for, in the present study. The animals were offered grass hay at ad libitum access. The hay contained (g/kg dry matter (DM)) organic matter, 926; crude protein, 72; neutral detergent fibre (NDF), 635; acid detergent fibre (ADF), 360 and gross energy, 16.5 (MJ/kg) as analysed in two subsamples by standard procedures (AOAC, 1997). Fresh water was available at all times. After 2 weeks of adaptation, intake (food offered and leftover) was registered daily, and faeces were collected completely for 7 days at regular intervals (from 4 h at the beginning up to 12 h on the last day). Faeces were dried to constant weight. These individual faecal samples were used for passage marker analysis (see below). From these samples, a representative pool sample was prepared for the analysis of faeces for DM, total ash, , crude protein, NDF, ADF and gross energy (AOAC, 1997). From these data apparent digestibilities of nutrients and energy were calculated as

(Intake – excretion) / intake × 100.

Mean ingesta retention times (MRT) were determined by feeding a particle (chromium-mordanted fibre, < 2 mm) and a fluid/solute (cobalt-EDTA) marker prepared according to Udén et al. (1980). Marker analysis followed the procedure outlined by Behrend et al. (2004) and Hummel et al. (2005); in doing so, wet ashing with sulphuric acid was followed by atom absorption spectroscopy. The MRT in the total gastrointestinal tract was calculated according to Thielemanns et al. (1978) as

$$MRT = \sum(t_i \times dt \times c_i) / \sum(dt \times c_i)$$

where t_i = time after marker application (h), dt = time interval represented by marker concentration (calculated as $(((t_{i+1} - t_i) + (t_i - t_{i-1})) / 2)$, and c_i = faecal marker concentration at time i (mg/kg DM). The marker was assumed to have been excreted completely once the faecal Co and Cr contents were the same as before marker application. The selectivity factor was calculated as $MRT_{particles}/MRT_{solutes}$. The indigestible gut content (V_N) and the total gut content (V) were calculated according to Holleman and White (1989) as

$$V_N = F * MRT$$

where F = faeces output (kg DM/h) and MRT = the average (2 mm) particle passage time through the entire digestive tract (h), and

$$V= (VN - (VN /(1 – (aD\ DM/100)))/\ln(1 – (aD\ DM/100))$$

assuming an exponential absorption of ingested food with time spent in the digestive tract. Because of the accepted linear scaling of gut fill with body mass (reviewed in Clauss et al., 2007b), gut fill was expressed as a proportion of body mass (BM).

Comparisons between rabbits and guinea pigs were performed using a t-test in PSAW 18.0 (SPSS Inc., Chicago, IL). The significance level was set to 0.05.

Results

On a metabolic body mass basis ($BM^{0.75}$), the rabbits tended ($p < 0.1$) to ingest less hay than the guinea pigs (Table 1). The rabbits apparently fed more selectively than the guinea pigs and the leftover of the hay offered was higher ($p < 0.05$) in NDF and ADF. The, rabbit faeces contained more ADF ($p < 0.05$) than guinea pig faeces, whereas their lower crude protein content was not significant. Consequently, fibre digestibilities were lower in the rabbits than in the guinea pigs; they also tended ($p < 0.1$) to express lower DM and OM digestibilities. The apparent digestibility of protein did not differ between the species. Whereas MRT of particles did not differ between the species, rabbits had drastically longer ($p < 0.001$) MRT of solutes than guinea pigs. The passage pattern of the markers showed a parallel movement of solute and particle markers in the guinea pigs, but a distinct separation between particles and solutes in the rabbits (Fig. 1). This pattern was consistent for all individuals of each species. Consequently, the calculated selectivity factor was very low in rabbits at 0.30 (95% confidence interval: 0.28 to 0.33) (Table 1). In guinea pigs, the selectivity factor was just above 1.0 (mean: 1.18, 95% confidence interval: 1.04 to 1.30). In both species, recurrent marker peaks were consistent with an assumed re-ingestion of the markers via coprophagy. The calculated DM gut fill was lower ($p < 0.01$) in the rabbits than in the guinea pigs.

Discussion

The fundamental differences in solute and particle passage patterns between rabbits and guinea pigs described previously for animals fed on pelleted compound feeds (Sakaguchi et al., 1987; Sakaguchi et al., 1992) are obviously also present in forage-only fed animals. The passage patterns as observed in the guinea pigs of the present experiment have been found in several

other rodent species with anatomical features of a ‘mucous-trap’ CSM (Pei et al., 2001). Recurrent marker peaks, considered typical for coprophagy (Clauss et al., 2007a), were evident in both species. The present experiment confirms previous findings on lower apparent digestibilities of DM and, in particular, fibre fractions in rabbits than in guinea pigs (Slade and Hintz, 1969; Sakaguchi et al., 1987; Sakaguchi et al., 1992). In contrast, there was no higher apparent crude protein digestibility and no lower protein contents in the hard faeces of rabbits as compared to that of guinea pigs as has been reported previously for rabbits in comparison with other rodents with a mucous-trap CSM (Slade and Hintz, 1969; González-Jiménez and Escobar, 1975; Sakaguchi, 2003). Furthermore, the general assumption that lagomorphs exhibit a particularly high protein digestibility (Monk, 1989) could not be corroborated by the present study, even though rabbit faeces contained numerically less crude protein than guinea pig faeces.

The present study illustrated that rabbits feed more selectively than guinea pigs, potentially due to their inherently lower capacity to digest fibre, and that rabbits have a higher DM digesta load than guinea pigs per unit body mass. A similar difference results when the data from Sakaguchi et al. (1987) on food intake, digestibility and particle retention of rabbits and guinea pigs on a pelleted compound feed are used to calculate DM gut fill (22.6 vs. 31.5 g/kg BM in rabbits vs. guinea pigs, respectively).

Measurements of a solute marker, such as Co-EDTA, are traditionally interpreted as ‘fluid retention’ or ‘fluid passage’ (e.g. Pickard and Stevens, 1972). Thus the pattern shown in Fig. 1 could be paraphrased as indicating a longer ‘fluid retention’ in rabbits than in guinea pigs. However, the interpretation that fluids are selectively retained in a ‘wash-back’ CSM is problematic. Clauss et al. (2010b) explained that retention times measured for fluid passage markers do not actually represent retention of fluid. In the passage of the digesta through the

gastrointestinal tract, fluid is constantly absorbed and excreted from and to the gut. The fluid excreted in the faeces therefore does not quantitatively represent a fraction of the fluid ingested via food or drinking water, but rather the last fraction of fluid excreted into the digesta and not absorbed from the distal colon. Because a fluid passage marker is, by definition, not absorbable, it is 'passed on' from one fluid fraction to the next. Excessive dosages of fluid passage markers can even lead to diarrhoea because the marker binds an excessive amount of water which remains in the intestinal tract (Bernard et al., 1995). Because the term 'fluid retention' presumably does not describe a physiological process, we advocate the use of the term 'solute retention', following Cork et al. (1999).

The behaviour of a solute marker, in comparison to the particle phase of digesta, represents the amount of fluid washing of that particle phase. The true importance of the solute marker may therefore consist in describing a type of washing which may be important to separate different digesta phases (Lentle et al., 2006) in order to enhance solute uptake at the luminal-intestinal border, or to separate very small particles (such as bacteria) from the total gastrointestinal contents. Secretion of fluids into, and washing of, the digesta can occur in both directions – aborad and orad. In many large mammalian herbivores, particularly in the grazing species, the MRT of solutes is often shorter than that of particles (Steuer et al., 2010), which indicates a particular washing of the particulate digesta phase with fluids in an aborad direction allowing the fluid marker to be transported faster than the particle marker. For ruminants, it has been suggested that this washing of the digesta with fluid in the forestomach leads to a particularly efficient harvest of microbes growing in the digesta (Clauss et al., 2010a). The 'wash-back' CSM of rabbits, with active fluid secretion in the proximal colon, retrograde fluid transport and fluid re-absorption in the caecum (Björnhag, 1972) probably has a similar effect in transferring

solutes and very small particles back into the caecum (Jilge, 1982). In analogy to ruminants, a retrograde flushing of the digesta might therefore be very useful to harvest microbes growing in the digesta plug.

Different from that fluid is constantly absorbed in the colon in guinea pigs, as is indicated by a monotonous increase in digesta DM content along the whole colon (Holtenius and Björnhag, 1985). Due to similar reported solute and particle retention patterns, a similar situation can be assumed for other caviomorph and myomorph rodents (Pei et al., 2001). It can be assumed that the 'mucus-trap' CSM is less efficient than the 'wash-back' CSM due to a slower extraction of bacteria from the digesta plug. This could translate into the necessity of a proportionately larger colon section in herbivorous rodents compared to lagomorphs to achieve a sufficient degree of bacteria extraction. This hypothesis that warrants investigation, but fits well to the comparatively lower DM gut loads calculated for rabbits. The distance to the groove is a crucial factor that determines the efficiency of protein extraction in the 'mucous-trap' CSM. This is obvious from findings in nutria showed that only the part of the colonic digesta plug that is close to the colonic groove is depleted of protein, whereas the digesta in the opposite portion of the plug retains a higher protein content (Takahashi and Sakaguchi, 2000). Still, the putative difference in efficiency between the CSM types need not necessarily – as suggested for example by Hörnicke (1981) – translate into a digestive advantage of the 'wash-back' CSM.

A slower, and potentially less complete, removal of bacteria from the digesta plug in a larger colon probably explains the higher digestibility of fibre from the same feed in guinea pigs and other herbivorous rodents as compared to rabbits, even though particle retention times are not distinctively different (Sakaguchi, 2003). The more selective feeding behaviour in rabbits, as found in this study, may be the response to counterbalance the lower capacity for fibre digestion.

If the 'wash-back' CSM of the lagomorphs is really associated with comparatively lower gut loads, it might help explain a peculiarity of this order: lagomorphs can run faster than other similar-sized mammals (Garland, 1983; Lovegrove, 2004). Apart from adaptations of metabolism and limb anatomy, a limited gut load (to reduce overall body mass) will contribute to this characteristic.

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Table 1 Mean (±SD) body mass, food intake, digestibility and methane production in rabbits and guinea pigs (n=6 per species).

Species	Rabbit	Guinea pig	p-value*
Body mass (BM, kg)	1.57 ± 0.31	0.79 ± 0.07	0.001
Dry matter (DM) intake (g/kg$^{-0.75}$ BM/day	50 ± 5	59 ± 11	0.076
Composition of ingested hay (g/kg DM)			
Organic matter	932 ± 2	937 ± 10	0.339
Crude protein	69± 2	69 ± 3	0.936
Neutral detergent fibre	613 ± 16	634 ± 2	0.025
Acid detergent fibre	331 ± 19	351 ± 7	0.054
Composition of leftovers (g/kg DM)			
Organic matter	889± 21	856 ± 33	0.065
Crude protein	82 ± 10	87 ± 10	0.462
Neutral detergent fibre	721 ± 21	642 ± 31	<0.001
Acid detergent fibre	477 ± 38	429 ± 33	0.043
Faeces composition (g/kg DM)			
Crude protein	99 ± 23	117 ± 10	0.112
Neutral detergent fibre	760 ± 66	733 ± 15	0.347
Acid detergent fibre	489 ± 09	468 ± 16	0.020
Apparent digestibility (proportion of intake)			
Dry matter	0.55 ± 0.06	0.61 ± 0.03	0.075
Organic matter	0.56 ± 0.06	0.62 ± 0.03	0.072
Crude potein	0.37 ± 0.09	0.35 ± 0.06	0.570
Neutral detergent fibre	0.44 ± 0.10	0.55 ± 0.05	0.038
Acid detergent fibre	0.34 ± 0.10	0.48 ± 0.06	0.014
Mean retention times (h)			
Particles	15 ± 3	18 ± 6	0.286
Fluids (Solutes)	51 ± 9	16 ± 4	<0.001
Selectivity factor	0.30 ± 0.03	1.18 ± 0.17	<0.001
Gut fill (g/kg BM)	19.6 ± 4.7	29.7 ± 4.1	0.003

*Independent sample t-test.

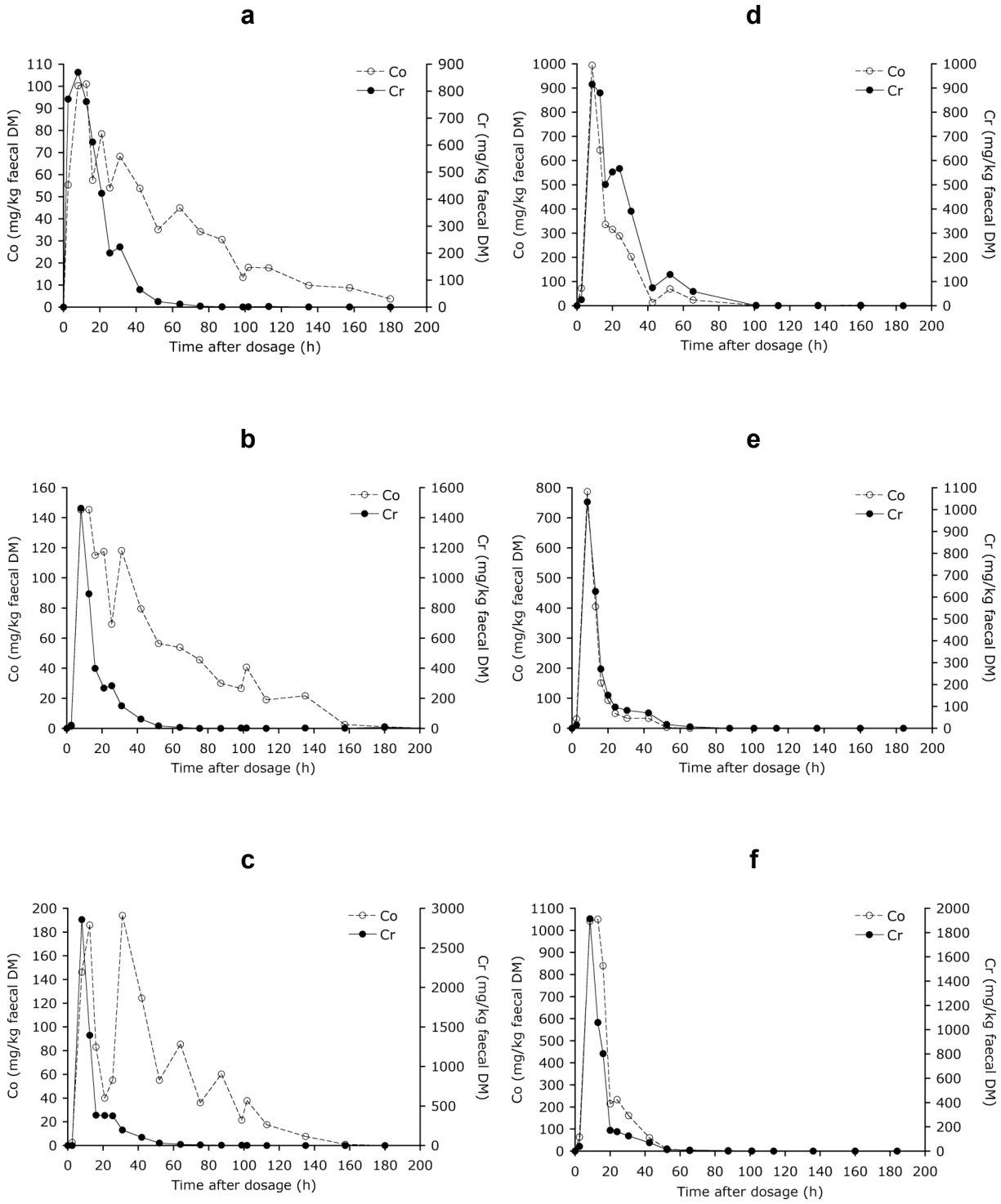

Figure 1 Faecal excretion pattern of solute (Co) and particle (Cr, < 2 mm) markers in three individual rabbits (a-c) and guinea pigs (d-f).

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